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Key message: **Major resistance gene to rhynchosporium, *Rrs18*, maps close to the telomere on the short arm of chromosome 6H in barley.**

Abstract

Rhynchosporium or barley scald caused by a fungal pathogen *Rhynchosporium commune* is one of the most destructive and economically important diseases of barley in the world. Testing of Steptoe x Morex and CIho 3515 x Alexis double haploid populations has revealed a large effect QTL for resistance to *R. commune* close to the telomere on the short arm of chromosome 6H, present in both populations. Mapping markers flanking the QTL from both populations onto the 2017 Morex genome assembly revealed a rhynchosporium resistance locus independent of *Rrs13*, that we named *Rrs18*. The causal gene was fine mapped to an interval of 660 Kb using Steptoe x Morex backcross 1 S_2 and S_3 lines with molecular markers developed from Steptoe exome capture variant calling. Sequencing RNA from CIho 3515 and Alexis revealed that only 4 genes within the *Rrs18* interval were transcribed in leaf tissue with a serine/threonine protein kinase being the most likely candidate for *Rrs18*.

Keywords: barley, *Rhynchosporium commune*, resistance, QTL, *Rrs18*, scald

Introduction

Rhynchosporium (scald) is one of the most destructive and economically important diseases of barley (*Hordeum vulgare* L.) worldwide, causing yield losses of up to 30-40%, particularly in parts of the world with a cool, wet climate (Avrova and Knogge 2012). Rhynchosporium also decreases grain quality, preventing an affected crop from being sold at a premium for malting (Shipton et al. 1974; Xi et al. 2000; Zhan et al. 2008). The disease is caused by the hemibiotrophic ascomycete *Rhynchosporium commune*. The life cycle and interaction of the fungus with barley is comprehensively described in a number of reviews (Zhan et al. 2008, Avrova and Knogge 2012).

Rhynchosporium is controlled by the use of fungicides, resistant cultivars and agronomic practices, with fungicides remaining the most common way of controlling fungal pathogens of cereals in developed countries. However, *R. commune* has a long asymptomatic phase, allowing very rapid development of disease when infection enters the symptomatic phase (Jenkins and Jemmett 1967; Davis and Fitt 1990). This makes chemical treatment difficult, and fungicide costs coupled with the evolution of fungicide insensitivity make chemical control an expensive requirement for growers (Oxley et al. 2003, Zhan et al. 2008). As such, effective cultivar resistance is an attractive option and resistance to this important disease has long been an important breeding target.

Although a number of resistance genes against rhynchosporium have been mapped in barley, none of them have been cloned. The 9 major resistance genes identified so far have been found on all chromosomes, except chromosome 5H (Zhan et al. 2008). A number of QTL have also been identified that map to regions of the chromosome with known major resistance genes, particularly at the centromeric region of 3H (*Rrs1*), the short arms of chromosomes 6 (6HS) (*Rrs13*) and 7 (7HS) (*Rrs2*) (Zhan et al. 2008). It has been suggested that some of these QTL are actually alleles of already identified major resistance genes (Bjørnstad et al. 2002; Wagner et al. 2008).

Many QTL have been identified based on data from field trials using natural inocula consisting of complex mixtures of *R. commune* genotypes. While such studies are useful for showing the effectiveness of resistance in the field, it makes it impossible to distinguish between partial and major gene mediated resistance. It also makes comparison of quantitative trait locus/loci (QTL) identified in mapping populations with known resistance genes difficult, and any comparison between genetic maps is further complicated by the use of different genetic marker sets (Zhan et al. 2008). It has been suggested that use of genetically monomorphic isolates of *R. commune* should provide reproducible results that can distinguish between major gene and partial resistance (Cheong et al. 2006).

A number of studies have attempted to identify *Rrs1* (Hofmann et al. 2013) and *Rrs2* (Hanemann et al. 2009; Marzin et al. 2016). Fine mapping of *Rrs2* has been hampered by a region of suppressed recombination colocating with the resistance gene, limiting physical resolution and preventing identification of a candidate gene (Hanemann et al. 2009; Marzin et al. 2016).

The major resistance gene *Rrs13* was first identified in a *H. vulgare* ssp. *spontaneum* x *H. vulgare* cultivar Clipper backcross (BC) 3 population (Abbott et al. 1991). The gene was subsequently mapped to chromosome 6HS, though the identified flanking markers used in these publications did not have published genetic map positions (Abbott et al. 1995; Genger et al. 2003). Many QTL have also been identified on 6HS

at a position similar to *Rrs13*. Cheong et al. (2006) identified a significant QTL in a Schooner x O'Connor double haploid (DH) population on 6HS. Wagner et al. (2008) identified a QTL on 6HS in a Post x Vixen DH population using *R. commune* isolate 271. A QTL on 6HS was also identified in an L94 x Vada population tested in the field by Shtaya et al. (2006). A single major resistance gene identified in a Vlamingh x WABAR2147 DH population by Wang et al. (2014) on 6H has flanking markers 1_1166 as distal marker and Bmag500 as proximal marker, clearly indicating the presence of a single major resistance gene outside the *Rrs13* interval. In most of these publications, markers are very sparse, and the use of different marker sets makes comparing the genetic maps difficult or impossible. Wagner et al. (2008) suggested that the QTL for adult plant resistance (APR) to rhynchosporium identified on 6HS could be alleles of *Rrs13*. The only successful comparison that has been carried out on 6HS resistance to rhynchosporium has been with the Schooner x O'Connor QTL in comparison to the *Rrs13* map produced by Genger et al. (2003). Here the authors suggested that there could be two resistance loci on 6H: *Rrs13* and a second locus closer to the distal end of the chromosome (Cheong et al. 2006).

The Spanish landrace CIho 3515 has outstanding resistance to rhynchosporium and has been found to contain two resistance loci (Habgood and Hayes 1971; Starling et al. 1971; Hofmann et al. 2013). The first resistance gene was found to be an allele of what was the *Rh-Rh3-Rh4* locus on chromosome 3H, now known as $Rrs1_{Rh4}$ (Hofmann et al. 2013). The second resistance gene was suggested as a new resistance gene named *Rh10* by Habgood and Hayes (1971) and proposed to be *Rrs13* by Hofmann et al. (2013).

The recent publication of a barley genome sequence, assembled into pseudomolecules, representing 7 chromosomes (Mascher et al. 2017), has allowed identification of highly accurate physical map positions for flanking markers of rhynchosporium resistance QTL, allowing comparison of marker positions from different genetic maps.

In this study, an analysis of barley resistance to rhynchosporium on 6HS was carried out using a CIho 3515 x Alexis (CxA) DH population, a Steptoe x Morex (SxM) DH population (Kleinhofs et al. 1993, Druka et al. 2008) and a SxM BC_1 population, using several genetically diverse isolates of *R. commune*. Unlike previous studies on rhynchosporium resistance, high coverage next generation sequencing data is now available for both Morex and Steptoe (Mascher et al. 2013; Mascher et al. 2017). The availability of exome capture sequence for Steptoe has further enabled identification of single nucleotide polymorphisms (SNPs) between Steptoe and Morex (Mascher et al. 2013). This allows rapid design of molecular markers for genotyping, making the SxM population ideal for the fine mapping of rhynchosporium resistance and identification of candidate resistance genes.

The aim of this study was to fine map a rhynchosporium resistance QTL identified at the telomeric region of 6HS in Steptoe. A comparison of the physical position of *Rrs13* and of QTL identified on 6HS in SxM and CxA DH populations revealed that these represent a single resistance locus independent of *Rrs13*. Comparison of RNA sequencing data for CIho 3515 and Alexis leaf tissue to sequence data available for Steptoe and Morex (Mascher et al. 2013; Mascher et al. 2017) led to identification of potential candidate genes for *Rrs18* and SNPs specific to Steptoe and CIho 3515, carrying *Rrs18*, compared to susceptible Morex and Alexis.

Materials and methods

Plant material and fungal isolates

For rhynchosporium resistance testing and QTL mapping studies, two DH populations consisting of 200 SxM DH lines and 245 CxA DH lines were used. Selected lines from the SxM BC_1 population developed by Phillips et al. (2015) were used for fine mapping of resistance on 6HS.

Two barley accessions, SBCC145 and Atlas, containing major rhynchosporium resistance genes *Rrs1*$_{Rh4}$ and *Rrs2* respectively, were used for comparison with the level of resistance of Steptoe and CIho 3515 (Table 1). In addition to the susceptible parents Morex and Alexis, barley cultivars Beatrix and Steffi were used as susceptible references for phenotyping using spray inoculation (Table 1).

R. commune single spore isolate L73a from the culture collection at the James Hutton Institute in Dundee (Scotland) and single spore isolates 271, UK7, LfL07, Rhy17, SGü4/3, S147-1 and Rhy174 from the collection held at the Bavarian State Research Centre for Agriculture in Freising (Germany) were grown on CZV8CM (Newton 1989) or lima bean agar medium (Rohe et al. 1996) at 17ºC in the dark. Fungal spores were harvested from 2-3 weeks old cultures by scraping the mycelial mat with a spatula following the addition of 5 ml of sterile distilled water (SDW). The tube containing spore suspension was vortexed for 30 s, after which the spore suspension was filtered through glass wool and centrifuged for 3 min at 1600 g. The resulting pellet was washed twice with 5 ml of SDW, followed by centrifugation at 1600 g for 3 min. The spore suspension was adjusted to a final concentration of 2-3 x 10^5 spores/ml for spray inoculation (Hanemann et al. 2009) or 1 x 10^5 spores/ml for spot inoculation of detached leaves (Newton et al. 2001).

All isolates were used to phenotype the parents of the 2 DH populations (Table 1). Isolates 271 and UK7 were used to phenotype 140 of 200 lines from the SxM DH population (Table S1) and isolates L73a and 271 were used for phenotyping the SxM BC_1S_2 lines. Isolates LfL07, S147-1 and Rhy174 were used to phenotype 238, 239 and 238 lines from CxA DH population respectively (Table S2).

Spray inoculation assay

A seedling spray inoculation assay was conducted as described in Schweizer et al. (1995). Briefly, four seeds per test line were sown in 6 x 6 cm² pots kept at 18°C for three days during germination and then at 16°C with 16 h light per day. Three weeks after sowing, plants at the 3-leaf stage were sprayed with a conidia suspension and kept at 16°C in the dark at 100 % humidity for 48 h. Subsequently, plants were kept at 16°C with 16 h day length. Symptoms were assessed on a 0-4 scale as described by Jackson and Webster (1976) with 0 representing no visible symptoms; 1 for very small lesions on the edge and the tip of the leaf; 2 for small defined lesions on the edge and the base of the leaf; 3 for big, confluent lesions on the whole leaf and 4 for total collapse and drying-out of the leaf. Four inoculated plants per line were scored individually at around 15 days post inoculation (dpi). The mean of the 4 scores was used as the rhynchosporium severity score for each line. Lines with a mean score of 2 and higher were considered to be susceptible for calculating segregation ratio of resistant to susceptible (R:S) lines for each dataset (Table S1, Table S2).

Detached leaf assay

Plants for detached leaf assays were grown for 2-3 weeks until the emergence of the 3rd leaf in a growth cabinet with a relative humidity (RH) of 75%, at 17°C under 16 h day length. Detached leaf assays were performed as described in Newton et al. (2001). Briefly, rectangular polystyrene boxes (79 x 47 x 22 mm) (Stewart Solutions, UK) were filled with approximately 20 ml of 0.5% water agar with 0.8 mM benzimidazole (Sigma, UK), retarding leaf senescence. Six 4 cm leaf segments from different lines were placed with the abaxial surface onto the set agar in each box. Leaves were brushed using a sable hair paintbrush to remove some of the cuticle waxes, to allow water droplets to stick to the leaf surface. The abraded area of each leaf was inoculated with 10 µl of spore suspension (10^5 spores/ml) and the boxes incubated in a controlled environment cabinet (Leec, model LT1201) at 17°C under 16 h day length, light intensity 200 lx s^{-1}. Each experiment included 6-10 replicate leaf segments for each line. Symptoms were observed from day 15 until day 28, and photographs were taken every 2-4 days. Lesion length was measured using ImageJ (Abramoff et al. 2004).

QTL mapping in SxM DH population

140 of 200 DH lines generated from a cross between barley cultivars Steptoe and Morex (Kleinhofs et al. 1993) were phenotyped previously by Druka et al. (2008) by spray inoculation using *R. commune* isolates 271 and UK7. Phenotypic data (Table S1) was obtained from www.genenetwork.org/webqtl/main.py.

Genotypes for 200 DH lines as well as the two parental cultivars (Table S1) were generated previously (Druka et al. 2008), using 1259 markers spread across the barley genome, and also obtained from www.genenetwork.org/webqtl/main.py. A genetic map was constructed from this genotypic data using the 'R/qtl' package (Broman, 2003) for R (R Core Team 2012). Marker phase was determined from the parental genotypes. Out of 1259 markers and 140 SxM lines, 180 markers and 6 lines were removed as more than 40 % of data points were missing. To improve the genetic map, a further 147 markers that deviated significantly ($p=<0.05$) from a 1:1 ratio were removed. Markers were assigned to linkage groups based on recombination fractions between pairwise marker combinations and the statistical significance of the recombination fraction as a logarithm of the odds (LOD) score. The maximum recombination fraction for placing two markers in the same linkage group was set at 0.33; the minimum LOD score for placing two markers in the same linkage group was set at 3.3. Linkage groups were matched to chromosomes based on previous marker assignments (Close et al. 2009). Marker order and positions were estimated using the 'orderMarkers' function in R/qtl package (Broman et al. 2003) from R (R Core Team 2012) with marker positions assigned using the Haldane mapping function. To improve the marker order on chromosome 3H, 21 markers were removed from the 3H linkage group so that the 3H marker order was roughly comparable to the consensus map of Close et al., (2009). 911 markers and 134 SxM DH lines were used to create the final genetic map (Table S1, Table S3).

QTL mapping was carried out using Genstat 17th edition (VSN International 2014) using the single trait/ single environment option. Genetic predictors were generated at 2 cM intervals. A simple interval mapping scan was carried out using these genetic predictors with the QSTLSCAN procedure. QTL candidates were identified as positions where the –log10(p) test statistic was higher than the threshold value that gave a genome wide error rate of 0.05. Genome wide error rate was calculated using the method described by Li and Ji (2005). Candidate QTL positions were selected using the QCANDIDATES procedure and used as cofactors in a composite interval mapping scan (again using the QSTLSCAN procedure).

Composite interval mapping was repeated until a stable set of candidate loci was identified. A final QTL model was then chosen based on these candidates. This was carried out using the QCANDIDATES and QMBACKSELECT procedure, which confirmed the significance of candidate QTL identified. Flanking markers were identified either side of a QTL peak by choosing the closest marker that had a –log10(p) score that was less than the peak –log10(p) by 1.5 (Table 2).

QTL mapping in the CxA DH population

Previously the Spanish landrace CIho 3515 has been found to contain two resistance loci: $Rrs1_{Rh4}$ on chromosome 3H (Habgood and Hayes 1971; Starling et al. 1971; Hofmann et al. 2013) and a second resistance gene on chromosome 6H proposed to be *Rrs13* by Hofmann et al. (2013). In order to investigate the resistance locus on chromosome 6H (whilst accounting for the effect of $Rrs1_{Rh4}$), markers were designed on both chromosomes. Primers designed using unigene expressed sequence tags (ESTs) were used to generate sequences from CIho 3515 and Alexis to create U35 and H35 SNP markers. SNP markers were converted to Cleaved Amplified Polymorphic Sequences (CAPS) markers using the program SNP2CAPS (Thiel et al. 2004) and used for genotyping. The CxA DH population was also genotyped using the 1536-SNP Ilumina GoldenGate OPA (Close et al. 2009) as described previously by Silvar et al. (2011). Additional markers used for genotyping included HVM0027 (Ramsay et al. 2000), STS_agtc17 (Grønnerød et al. 2002; Patil et al. 2003), HVM0060 (Patil et al. 2003), 11_0205 (Hofmann et al 2013), 11_1476 (Hofmann et al 2013), Falcon (Penner et al. 1996), available SSR markers (Ramsay et al. 2000; Li et al. 2003; Rostoks et al. 2005; Stein et al. 2007; Varshney et al. 2007) and SNPs (Rostoks et al. 2005; Stein et al. 2007) for chromosomes 3H and 6H.

Genetic map construction was carried out using the R/qtl package as described for the SxM DH population. The maximum recombination fraction for placing two markers in the same linkage group was set at 0.4, the minimum LOD score for placing two markers in the same linkage group was set at 4. Linkage groups were assigned chromosomes based on previous published information (Ramsay et al. 2000; Li et al. 2003; Rostoks et al. 2005; Stein et al. 2007; Varshney et al. 2007).

Markers and their positions from the linkage groups on 3H and 6HS (Table S4) were used for QTL mapping. QTL mapping was carried out as described for the SxM DH population, using genotypic data and average disease scores for each of 238, 239 and 238 lines from CxA DH population spray-inoculated with isolates LfL07, S147-1 and Rhy174 (Table S2).

DNA extraction

Barley genomic DNA for SxM populations was extracted from the youngest leaf of 3-4 weeks old plants on a Qiagen QIAcube HT/QIAxtractor platform (Qiagen, UK) using standard operating procedure. To test quality and concentration, 5 µl of extracted DNA were run on a 1% agarose gel and band intensity was compared to GeneRuler 1Kb Ladder (Thermo Fisher Scientific, UK). DNA concentration was also measured using a NanoDrop spectrophotometer (NanoDrop Technologies Inc., USA) or Quant-iT PicoGreen (Invitrogen, UK).

For the CxA DH population genomic DNA was isolated from frozen barley leaves using a NucleoSpin Plant II Minikit (Macherey-Nagel GmbH & Co. KG, Germany) or according to Behn et al. (2004).

KASP genotyping

Kompetitive allele specific PCR (KASP) primers (Table S5, Table S6) were designed around the SNPs within the *Rrs18* interval based on oligonucleotide pool assay (OPA) markers or Steptoe exome capture variant calling data (Mascher et al. 2013). DNA sequence containing 70 bp each side of the SNP was used for designing two allele-specific and a conserved primer for each KASP assay using a custom python script. BLASTn comparison, using the default settings, was carried out against the 2012 Morex genome assembly (IBSC 2012) to determine if the sequence was unique.

Eight µl reactions were prepared in MicroAmp Fast optical 96-well plates (Fisher Scientific, UK) using <3 ng of DNA, 4 µl of 2x KASP reagent (LGC, UK), two allele-specific primers at 0.16 µM each and a conserved primer at 0.4 µM. PCR and genotyping was completed using a StepOne Plus real-time PCR machine (Applied Biosystems, USA), with the KASPar 55 plus 6 step program. Sample fluorescence was measured at 20°C for 2 min, then DNA was denatured for 15 min at 94°C, followed by 10 cycles of 20 s at 94°C and 1 min at 62°C (decreasing by 0.7°C per cycle). This was followed by 32 cycles of 20 s at 94°C and 1 min at 55°C. Samples were then cooled to 20°C for 2 min to allow fluorescence measurement.

BeadXpress genotyping

Barley genomic DNA concentration was adjusted to 50 ng/µl with Tris–EDTA, pH 8.0. 500 ng of genomic DNA was used for a 384 SNP Illumina GoldenGate OPA using the BeadXpress platform (Illumina Inc., UK) according to the manufacturer's protocol. A set of 384 SNPs distributed along the entire length of chromosome 6HS, including SNPs selected from previously published sources (Close et al. 2009) as well as SNPs identified by resequencing barley ESTs, in a range of lines (including cultivars Steptoe and Morex) was used. Briefly: barley ESTs from the HarvEST assembly 35 (http://www.harvest-web.org/) that were predicted to map to barley 6HS by homology with rice gene models located on the syntenous region of rice chromosome 2H (Mayer et al. 2011) were aligned to the corresponding rice gene model to identify potential introns. Primers were designed across predicted introns and PCR products sequenced using an Applied Biosystems AB3730 sequencer to identify polymorphisms. Allele calls were performed using the "GenTrain" clustering algorithm available in Genome Studio v2011.1 (Illumina Inc., UK). Each SNP-call was checked manually in Genome Studio for quality and accuracy. The 384 OPA markers data were filtered to remove markers monomorphic for Morex and Steptoe, and failed markers leaving 64 OPA markers within the part of 6HS containing the *Rrs18* region for mapping (Table S7).

Mapping *Rrs18* in SxM BC_1S_2 lines

Selected lines from a SxM BC_1 population developed by Phillips et al. (2015) were phenotyped with *R. commune* isolates L73a (detached leaf assay) and 271 (spray inoculation) and genotyped with 64 OPA (Table S7) and 2 exome capture-based SNP makers (Table S6, Table S8).

For *R. commune* isolate 271 the average symptom score 17 dpi was used as the phenotype for each line. A 2-tailed t test of association was carried out for the phenotypes for each marker allele. LOD scores were generated from the resulting P values, by converting the P values into a Likelihood ratio score (LRS). The LRS was then converted to a LOD score: LOD=LRS/(2 x ln10). The LOD score and physical position for each associated marker were plotted (Fig. 4a). The peak LOD for isolate 271 phenotypes -1.5 (a 1.5 LOD drop) was used to identify 95% confidence flanking markers. Statistical analysis was carried out using R (R Core Team 2012).

In the case of L73a, the greatest average lesion size was calculated for each line. The list of scores was used for a 2-tailed t test of association for each marker allele. P values were converted to LOD scores (as described above) and the LOD score and physical position for each marker were plotted (Fig. 4b-c).

Further phenotyping was carried out on 8 additional and 9 of 24 previously used genotyped SxM BC_1S_2 lines using a detached leaf assay with *R. commune* isolate L73a with up to 10 leaf replicates per line. The phenotypes from this experiment were combined with the L73a phenotypes of the previous mapping using a REML model. As before the greatest lesion size for each replicate was used in subsequent analysis. Lines were genotyped with 10 KASP markers across the *Rrs18* interval and marker 11_10165 at position chr6H_14306329 (Table S5, Table S6, Table S9). To analyse the data, phenotypes and genotypes from these 17 lines were combined with the data for the 24 SxM BC_1S_2 lines used for mapping previously. 11 marker genotypes for 24 SxM BC_1S_2 lines were predicted based on previous genotyping (Table S8). Marker associations with average greatest lesion size were tested using R package 'lme4' (Bates et al. 2015) by comparing a null REML model (fixed terms: Experiment, random terms: Family, Line, Box) with the same model but incorporating the marker allele as a fixed term using the 'anova.lmerModLmerTest' function of the 'lmerTest' package (Kuznetsova et al. 2017) using default argument values. P values were converted to LOD scores as previously described. The interval for the QTL was determined from a 1.5 LOD drop from the highest marker LOD score. Phenotypes were permuted 100 times and the 5th highest LOD was used as the 0.05 error rate.

Identification of physical positions of markers associated with *Rrs18* in barley genome sequence

Sequence comparison using the IPK BLAST server (http://webblast.ipk-gatersleben.de/) on default settings was carried out using the primer sequences of markers associated with rhynchosporium resistance QTL on 6HS, and flanking markers for *Rrs13* in BC line 30 (Genger et al. 2003) and AB30 (Cheong et al. 2006). The August 2015 Barley pseudomolecule contigs were used as the subject of the BLASTn search. In all cases, the best hit was used to determine the physical position of the sequence matching the primer.

In the case of the 64 OPA SNPs, BLASTn search with the default settings was used with the sequence manifest for each SNP against the 6HS barley pseudomolecule.

Phenotyping of heterozygous SxM BC_1 lines using isolate L73a

To determine whether the resistance caused by *Rrs18* is dominant, 6 SxM BC_1S_1 lines predicted to be heterozygous at *Rrs18* locus were chosen, and 20 S_2 seeds of each line were planted. For the purpose of analysis in order to calculate background effects, 2 out of the 6 S_1 lines chosen were selfed from the same SxM BC_1 line, while the other 4 were selfed from another SxM BC_1 line. S_2 lines could be part of the same

family (share the same SxM BC_1 parent) or the same subfamily (share the same SxM BC_1 S_1 parent). Lines that grew were genotyped with KASP markers chr6H_10925141 and chr6H_11571800 (Table S6). All genotyped progeny from each of the 6 BC_1S_1 lines were tested with a goodness of fit test for expected genotype ratio of 1:2:1. All 6 BC_1 S_1 lines were found to have progeny that did not significantly deviate from the expected 1:2:1 genotype ratio (p=>0.05). Three SxM BC_1 S_2 lines with Steptoe, 3 with hetrozygous and 3 with Morex genotypes at *Rrs18* were chosen from each subfamily. Five leaf replicates all from the same leaf per line were phenotyped with *R. commune* isolate L73a using a detached leaf assay. In most cases the 3rd leaf was used. Leaves were photographed at 14, 18, 22 and 26 dpi and images analysed using ImageJ (Abramoff et al. 2004). A REML analysis was carried out using the R package lme4, with the linear model ~ Genotype + (Family\Subfamily\Line) used to predict means. Genotype was fitted as a fixed effect while (Family\Subfamily\Line) were included as random effects. The R package 'predictmeans' (https://cran.r-project.org/web/packages/predictmeans/predictmeans.pdf) was used to calculate the average Least Significant Difference (LSD), to determine whether differences between genotype means were significant (Table 3).

Sequencing of RNA from CIho 3515 and Alexis leaves and variant calling

R. commune strain T-R214-GFP (Thirugnanasambandam et al. 2011), expressing green fluorescent protein (GFP), was used for inoculation of CIho 3515 and Alexis leaves, which allowed to confirm infection at 3 dpi. Second leaves of 3 weeks old CIho 3515 and Alexis plants were laid flat and gently rubbed with a paint brush prior to spot inoculation with 10 µl drops of spore suspension (2 $x10^4$ spore/ml) with ~ 15 mm gaps between drops. Plants were kept at 100% humidity for 3 days, at 18ºC, with the first 24 h in dark. Leaf samples for RNA extraction were taken at 3 dpi.

Total RNA was extracted using the Qiagen RNeasy Plant Mini kit (Qiagen, UK) following the protocol supplied by the manufacturer. RNA concentration was estimated using a NanoDrop spectrophotometer (NanoDrop Technologies Inc., USA). RNA quality was assessed using a bioanalyser (Agilent Technologies, USA). One µg of RNA was used for TruSeq® RNA Sample Preparation following the manufacture's protocol and sequencing was conducted on an Illumina NextSeq 550 System (Illumina Inc., UK).

Total read counts for the RNA-seq samples of CIho 3515 and Alexis were 69,273,356 and 77,007,618 respectively (2 x 76 bp paired end reads). The reads were mapped to the barley reference sequence (Mascher et al. 2017) using the splice mapping software STAR v. 2.5.3a (Dobin et al. 2013) with the parameters "--twopassMode Basic--outBAMcompression 10 --outFilterMismatchNmax 1 --outFilterMatchNminOverLread 0.97". This set of parameters allows a single mismatch per read and a maximum of two per read pair and reduces mismapping-related false positive SNPs to a minimum (Ribeiro et al. 2015).

Variants in the *Rrs18* interval (10,904,998-11,579,918 bp) were called using the UnifiedGenotyper component from the Genome Analysis Toolkit v. 3.4.0 (GATK) (McKenna et al. 2010), using default settings, but with three additional flags required for a) spliced mappings ("-U ALLOW_N_CIGAR_READS") and b) reassignment of MAPQ values to 60 as STAR does not output these

("-rf ReassignMappingQuality -DMQ 60"). SNP effect annotation was carried out using the SnpEff tool (Cingolani et al. 2012). Visual spot checks of mappings and variant calls were carried out using Tablet (Milne et al. 2010a; Milne et al. 2013).

The variant calls from CIho 3515 and Alexis were combined with a set of variant calls obtained for Steptoe, which were based on previously published (Russell et al. 2016) exome capture data (European Nucleotide Archive, accession number ERS243312, https://www.ebi.ac.uk/ena/data/view/ERS243312). Read mapping for this line was carried out in line with the GATK Best Practices pipeline (Van der Auwera et al. 2013), using BWA (Li and Durbin 2009) and the GATK v. 3.4.0 HaplotypeCaller. Details of the mapping and variant calling approach are published elsewhere (Bayer et al. 2017).

The VCF files containing the calls from CIho 3515 and Alexis and that containing the Steptoe calls were merged using the GATK's CombineVariants tool, and our own custom Java code was then used to further subset this file to only retain SNPs where

- there were exactly two alleles present
- there were no missing data
- CIho 3515 and Alexis had different alleles

The VCF file with the remaining SNPs was then converted using custom Java code and visualized with the Flapjack software (Milne et al. 2010b).

Results

Disease resistance

The rhynchosporium-resistant cultivar Steptoe and line CIho 3515, along with barley line SBCC145 and cultivar Atlas (containing major rhynchosporium resistance genes *Rrs1*$_{Rh4}$ and *Rrs2* respectively), and four highly susceptible cultivars, Morex, Alexis, Beatrix and Steffi, were tested for resistance to 9 different *R. commune* isolates 271, UK7, R214, Rhy174, S147-1, LfL07, SGü4/3, Rhy17 and L73a (Table 1). The first 8 isolates were individually used in spray inoculation of 3 weeks old barley plants while isolate L73a was used for inoculation of detached leaves. Barley landraces SBCC145 and CIho 3515 were highly resistant to all *R. commune* isolates used in this study apart from isolate L73a which caused smaller lesions on CIho 3515 compared to susceptible cultivars Morex and Alexis (Table 1). Most isolates did not cause any symptoms on SBCC145 and CIho 3515 and isolates 271, UK7, Rhy174 and LfL07 caused very small lesions on the edge and the tip of some leaves resulting in mean infection scores ranging from 0.1 to 0.3 (Table 1). Most of the *R. commune* isolates tested were able to partially (in the case of isolates 271 and UK7) or completely (in the case of isolates R214, Rhy174, S147-1, LfL07, SGü4/3 and Rhy17) overcome *Rrs2* resistance in cultivar Atlas. At the same time, cultivar Steptoe was resistant to *R. commune* isolates R214, Rhy174, S147-1 and LfL07, with mean infection scores ranging from 0.1 to 1.3. Cultivar Steptoe was also highly resistant to isolates 271 and UK7, with mean infection score of 0.9 and 0 respectively, moderately susceptible to isolate SGü4/3, with mean infection score of 2.0, and highly susceptible to isolate Rhy17, with mean infection score of 4.0. Cultivars Alexis, Beatrix and Steffi were susceptible to all isolates tested, reaching mean infection

scores of 2.9 - 4.0, while cultivar Morex was susceptible to all isolates tested, with mean infection scores of 2.8 - 4.0, apart from isolate R214 causing mean infection scores of 1.3 (Table 1). Isolate Rhy17 was recognised by barley landraces SBCC145 and CIho 3515 containing *Rrs1*$_{Rh4}$, but not by Steptoe, suggesting that Steptoe does not have *Rrs1*$_{Rh4}$. These results suggested that cultivar Steptoe might contain an *R* gene different to *Rrs1*$_{Rh4}$ and *Rrs2*.

Previously a population of 200 DH lines derived from a cross between cultivars Steptoe and Morex was used to generate mRNA transcript abundance, trait and genotypic data sets (Druka et al. 2008). 140 lines from this mapping population were assessed for resistance to *R. commune* isolates 271 and UK7 (Fig. 1). Mean disease scores for parental lines were on average 1.9 for Steptoe and 2.4 for Morex with isolate 271, and 0.0 for Steptoe and 3.1 for Morex with isolate UK7 (Fig.1a-b). Mean disease scores for the population were 2.2 and 1.0 for isolates 271 and UK7 respectively (Fig.1a-b). Phenotyping with isolate 271 resulted in a 1:1.7 ratio of resistant and susceptible lines, suggesting that more than one resistance gene was segregating. With isolate UK7 however, the resistant to susceptible (R:S) ratio was 3.3:1, with most lines having a disease score of less than 1, suggesting the presence of 2 resistance loci in the population, both conveying full resistance and both segregating 1:1 (Expected segregation ratio (R:S) =3:1; χ^2=0.17, p=0.68).

Another population used in this study was the CxA DH population, developed to characterise CIho 3515 resistance to rhynchosporium. The CxA DH population showed highly differential response to 3 *R. commune* isolates LfL07, S147-1 and Rhy174. The response to isolates LfL07 and S147-1 was characterized by a disproportionally high number of fully resistant lines and very few lines with an intermediate reaction, especially in case of isolate S147-1 (Fig. 2a-b). This led to population mean disease scores of 1.1 and 0.8 for isolates LfL07 and S147-1 respectively (Fig. 2a-b). Inoculation with isolate Rhy174 resulted in very few DH lines without disease symptoms (Fig. 2c). A high number of lines displayed medium resistance with scores between 1 and 2, and about half of the DH lines were fully susceptible with a score of 4, resulting in the population mean disease score of 2.6 (Fig. 2c). Phenotyping with isolates LfL07 and S147-1 resulted in a 3.3:1 and 4.2:1 R:S ratio respectively, suggesting presence of more than 1 resistance loci in the population (Expected segregation ratio (R:S) =1:1; χ^2=66.7, p<0.01; χ^2=90.4, p=0.04). In the case of isolate Lfl07 there is strong support for the presence of two resistance genes, each segregating 1:1 and conferring complete resistance (Expected segregation ratio (R:S) = 3:1 χ^2=0.27, p=0.6) (Table 2, Fig. 2c). Phenotyping with isolate Rhy174 resulted in an approximately 1:1 ratio of resistant and susceptible lines, implying that one locus is active conveying partial resistance (Expected segregation ratio (R:S) =1:1; χ^2=3.3, p=0.07) (Table 2, Fig. 2c).

Mapping rhynchosporium resistance loci in SxM and CxA DH populations

A single environment QTL analysis was carried out on SxM DH population using average disease scores after infection with *R. commune* isolates 271 and UK7, and a genome wide significance threshold of 0.05. In the case of isolate 271, 4 significant QTL were identified on chromosomes 3H, 6H and 7H (Table 2, Fig. 1c). The largest QTL on 6H, qS271_6a explained 30% of the phenotypic variation, with Steptoe providing the resistant allele (Table 2, Fig. 1c). QTL qS271_3, that mapped to the centromeric region of 3H, explained 12.6 % of the phenotypic variation (Table 2). The minor QTL qS271_6b explained 9.6 % of the phenotypic variation; Morex provided the resistant allele (Table 2, Fig. 1c). Another minor QTL qS271_7 explained 7.4

% of the phenotypic variation, with Steptoe providing the resistant allele (Table 2, Fig. 1c). With isolate UK7, two highly significant QTL were identified on 6H and 3H (qSUK7_6, qSUK7_3), with a minor QTL on 5H (qSUK7_5) which explained 6.9% of the phenotypic variation (Table 2, Fig. 1d). QTL qSUK7_6 and qSUK7_3 explained 41 % and 30.4 % of the total phenotypic variation respectively, with Steptoe providing the resistant allele in both cases (Table 2, Fig. 1d). qSUK7_6 mapped between 7.89 and 12.01 Mb, which is within the interval for qS271_6a (Table 2). qSUK7_3 at 201.16 – 508.77 Mb includes the *Rrs1*$_{Rh4}$ region, defined by flanking markers 11_0010 and 11_0823 (Hofmann et al. 2013) at 489,991,522 and 491,895,585 Mb respectively (Looseley et al. 2018). It is at a different position from the less significant QTL qS271_3, which is located between 591.89 and 617.76 Mb on chromosome 3H (Table 2). Furthermore, the resistant allele for qSUK7_3 is from Steptoe, while the resistant allele for qS271_3 comes from Morex (Table 2).

The QTL analyses identified 2 major loci in CIho 3515 contributing considerably to the resistance to isolates LfL07, S147-1 and Rhy174, one on chromosome 3H and one on chromosome 6H (Fig. 2d). The dominating source of resistance to isolates LfL07 and S147-1were the QTL qC07_3 and qC147_3 on chromosome 3H, explaining 63.7 and 59.5 % of the phenotypic variation, with the QTL qC07_6 and qC147_6 on chromosome 6H contributing 6.3 and 11.7 % of the phenotypic variation respectively (Table 2). The dominating source of resistance to isolate Rhy174 was the QTL qC174_6 explaining 68.9 % of the phenotypic variation, whereas the QTL qC174_3 contributed only 3.9 % of the phenotypic variation (Table 2, Fig. 2d). qC07_3 and qC147_3 at 457.98 - 542.28 Mb, and qC174_3 at 457.98 - 557.36 Mb include the *Rrs1*$_{Rh4}$ region (Table 2).

Physical positions of rhynchosporium resistance loci on 6HS

Physical positions on the Morex pseudomolecule 6HS (Mascher et al. 2017) were identified for markers Cxp3, BMag500 and MWG916, the closest available flanking markers for *Rrs13* (Genger et al. 2003; Cheong et al. 2006), and for the flanking markers 2_0262 and 1_1479, and U35_24165 and U35_40281 for the resistance QTL identified on 6H in the SxM and CxA DH populations respectively.

The flanking markers for the major resistance gene *Rrs13* were found to be at a different locus to rhynchosporium resistance QTL identified on 6H in the SxM and CxA DH populations (Fig. 3). The flanking markers most closely associated with *Rrs*13 (Cheong et al. 2006) had matching sequence at 16.14 and 29.10 Mb on the Morex pseudomolecule 6HS. The flanking markers identified for the resistance locus present in the CxA DH population were mapped to 10.01 and 12.05 Mb respectively (Table 2, Fig. 3). This overlapped with the SxM resistance locus at 7.89 to 12.01 Mb (Table 2, Fig. 3).

Further mapping of rhynchosporium resistance QTL from Steptoe on chromosome 6HS

To further map rhynchosporium resistance QTL on 6HS 284 lines containing both monomorphic and polymorphic markers between OPA markers 11_10669 and 11_10023 (Close et al. 2009), mapped to 3.9 and 24.4 cM on 6HS respectively (Table S3), were selected from a SxM BC_1 population developed and originally genotyped by Phillips et al. (2015). The selected lines were selfed, grown and genotyped with 4 OPA markers: 11_10669, 11_21032, 11_10165 and 11_10023 (Close et al. 2009) (Table S3, Table S5). Seeds from 24 SxM BC_1S_2 lines that were found to be homozygous between 3.9 and 24.4 cM on 6HS were grown for phenotyping with *R. commune* isolates 271 (spray inoculation) and L73a (detached leaf assay) and

genotyping with 64 OPA and 2 exome capture based SNP makers (Table S8). 18 lines were known to contain a Steptoe introgression, while 6 chosen lines known to carry only Steptoe or Morex alleles were used as controls. Both isolates were able to cause disease symptoms on resistant lines (Table S8). Susceptible controls infected with *R. commune* isolate 271 did not reach the expected maximum score of 4, suggesting that the pathogen has lost some of its ability to cause infection. Line SM_BC1_FM_25_10_3 carrying the resistant Steptoe allele had a moderately resistant score when inoculated with isolate 271, while large lesions formed on the Steptoe allele carrying line SM_BC1_MF_15_07_06 following inoculation with isolate L73a (Table S8). Mean infection score with isolate 271 was 0.9 and 2.3 for resistant and susceptible lines respectively (Table S8), in agreement with previous average scores for Steptoe and Morex with isolate 271, Table 1, Table S1, Fig.1a). Mean lesion size with isolate L73a was 8.5 and 12.7 mm for resistant and susceptible lines respectively (Table S8). The resistance locus on 6HS accounted for 75 % and 52 % of the total phenotypic variation when using isolate 271 and L73a respectively. Mapping with isolate L73a gave a 1.5 LOD support interval between 9.19 and 13.88 Mb, while mapping with isolate 271 resulted in a slightly higher resolution with 1.5 LOD support interval of 9.08 and 11.78 Mb on 6HS (Fig. 4a-b).

To find additional lines suitable for fine mapping, seed from SxM BC_1 lines (Philips et al. 2015) that had a recombination event between OPA markers 11_21032 and 11_11479 (Fig. 5), were grown (SxM BC_1S_1) and genotyped using markers chr6H_ 9620201 and chr6H_ 12057992 (Table S6). Seed from 8 lines which were homozygous for the previously identified recombination were grown for further fine mapping. To further narrow down the *Rrs18* interval 9 additional KASP markers were designed within the 9.62-12.57 Mb interval to allow identification of SxM BC_1S_2 lines with additional recombination events (Fig. 5, Table S6, Table S9). The final mapping experiment was based on genotypes and average greatest lesion size, following inoculation with *R. commune* isolate L73a, for the set of 30 SxM BC_1S_2 lines (Table S9). Recombination between KASP markers chr6H_10925141 and chr6H_11264412 was detected in 2 susceptible lines: SM_BC1_FM_15_23_2_2_3 and SM_BC1_FM_15_23_3_16 (Fig. 5, Table S9). Recombination between KASP markers chr6H_11572955 and chr6H_11581565 was detected in another 2 lines: resistant line SM_BC1_MF_15_12_04 and susceptible line SM_BC1_MF_15_13_01_19 (Fig. 5, Table S9). No further recombination has been detected between 3 KASP markers, chr6H_11264412, chr6H_11571800 and chr6H_11572955 (Fig. 5, Table S9). This mapping put the *Rrs18* interval between 10.96 and 11.58 Mb on 6HS (Fig. 4c). The interval calculated by 1.5 LOD drop corresponds to recombination events at around 10.92 and 11.58 Mb, which is within the interval defined by KASP markers chr6H_10925141 and chr6H_11581565 (Fig. 4c, Fig. 5, Table S9). This estimate gave an interval for *Rrs18* of approximately 660 kb, according to the latest barley genome assembly of cultivar Morex (Mascher et al. 2017).

Testing of *Rrs18* dominance

The vast majority of plant *R* genes are dominant. In order to determine whether the *Rrs18* resistance is also dominant a detached leaf assay with the *R. commune* isolate L73a was carried out on SxM BC_1S_2 lines with a genotype in the *Rrs18* region of either homozygous Steptoe, homozygous Morex or heterozygous. 17 lines of each genotype were included. Lines with a homozygous Morex genotype were found to have significantly larger lesions than those with a homozygous Steptoe genotype (Table 3), consistent with the previously

described mapping experiments. Lines which were heterozygous at *Rrs18* had a mean maximum lesion size significantly smaller than those with the homozygous Morex genotype, but not significantly different from those with the homozygous Steptoe genotype. This result strongly suggests that *Rrs18* is dominant.

Genes within *Rrs18* interval and *Rrs18*-specific SNPs

The 660 kb *Rrs18* interval contains 11 high confidence and 10 low confidence genes according to the latest Morex genome annotation (Mascher et al. 2017). At closer examination 4 out of 10 low confidence genes appeared misannotated and were removed, leaving a total of 17 genes (Table 4, Fig. 5). HORVU6Hr1G005120 was annotated as an F-box domain protein, though conflicting information in the original annotation suggests it might be a transposable element. HORVU6Hr1G005150 and HORVU6Hr1G005170 were annotated as two-component response regulator ARR11, while they match hornerin-like protein, with a BLASTx e-value of 8e-29 and 7e-28 respectively (Table 4).

Reads for only 4 genes within the *Rrs18* interval were present in the RNA-seq data for both CIho 3515 and Alexis (Table 4-5, Fig. 5), suggesting that the remaining genes are not transcribed in leaves of barley seedlings. All of the transcribed genes were annotated as high confidence genes (Mascher et al. 2017). They include HORVU6Hr1G005080, annotated as elongation factor P; HORVU6Hr1G005240, annotated as pentatricopeptide repeat-containing protein; HORVU6Hr1G005250, annotated as an allene oxide synthase and HORVU6Hr1G005260, annotated as a protein kinase (Table 4). HORVU6Hr1G005260's predicted protein sequence contains a potential extracellular domain with a signal peptide, a transmembrane domain and a serine/threonine kinase domain, making it the most likely candidate for the *Rrs18* (Table 4).

Given the similarity in map position of *Rrs18* in SxM and CxA DH populations (Fig. 1-2), it is highly likely that resistance on 6HS is caused by the same gene in Steptoe and CIho3515. If the difference in phenotype is caused by a variant or variants present in one of the candidate genes, those variants should be shared between CIho 3515 and Steptoe, and absent in susceptible lines. To compare the 4 parental lines, variants were identified between CIho 3515 and Alexis in *Rrs18* region (10.92 - 11.58Mb) and compared to alleles in Steptoe and Morex (Table 5). All 4 genes with RNA-seq reads contained variants between CIho 3515 and Alexis, with 19 SNPs in total identified within genes in this region (Table 5). Six out of these 19 variants had the same allele present in CIho 3515 and Steptoe, with the alternative allele present in Alexis and Morex. One of these SNPs was present in putative elongation factor P HORVU6Hr1G005080, 4 SNPs were found in the putative allene oxide synthase HORVU6Hr1G005250 and 1 SNP in putative protein kinase HORVU6Hr1G005260. Only 2 out of 6 SNPs differentiating between CIho 3515 and Steptoe, and Alexis and Morex could result in non-synonymous substitution: chr6H_11518293 in putative allene oxide synthase HORVU6Hr1G005250, leading to a change from leucine to valine, and chr6H_11571800 in putative protein kinase HORVU6Hr1G005260, leading to a change from threonine to alanine (Table 5). Additional analysis of these two SNPs with PROVEAN (http://provean.jcvi.org/seq_submit.php) revealed that in both cases the amino acid substitutions are neutral and have no deleterious effect on the protein.

Discussion

Previous studies have identified multiple QTL conferring resistance to rhynchosporium on the distal end of chromosome 6H (Jensen et al. 2002; Cheong et al. 2006; Shtaya et al. 2006; Wagner et al. 2008; Wang et al. 2014). While Cheong et al. (2006) suggested that rhynchosporium resistance identified on 6HS in a Schooner/O'Connor population could be a new locus independent of *Rrs13,* due to the difficulty of comparing genetic maps and sparsity of molecular markers it was not clear whether this and other QTL represented alleles of *Rrs13* or an entirely different resistance locus.

Previously Spanish landrace CIho 3515 has been found to contain two resistance loci: $Rrs1_{Rh4}$ and a second resistance gene proposed to be *Rrs13* by Hofmann et al. (2013). In this study the QTL analyses also identified 2 resistance loci in CIho 3515, one containing the *Rrs1*interval on chromosome 3H and one on chromosome 6H (Table 2, Fig. 2d).

The initial testing of barley cultivar Steptoe for resistance to *R. commune* isolates with different race specificities showed that while it was fully or partially resistant to most of the *R. commune* isolates used, it was highly susceptible to isolate Rhy17, which was recognised by barley landraces SBCC145 and CIho 3515 containing $Rrs1_{Rh4}$, suggesting that Steptoe does not have $Rrs1_{Rh4.}$, but might contain a different *R* gene. The QTL analysis carried out on SxM DH population following inoculation with *R. commune* isolates 271 and UK7 showed that the biggest effect, explaining 30% and 41 % of the phenotypic variance, respectively, was explained by the QTL on 6HS, with Steptoe providing the resistant allele (Table 2, Fig. 1c).

The availability of a near complete reference barley genome sequence (Mascher et al. 2017) has enabled identification of physical map positions of a variety of genetic markers, allowing a direct comparison between independent genetic maps. Flanking markers for resistance identified in the SxM and CxA DH populations on 6HS were located at 7.89 and 12.01, and at 10.01 and 12.05 Mb respectively (Table 2), while the most recent flanking markers for *Rrs13*, identified by Cheong et al. (2006), mapped to the 6H pseudomolecule at 16.14 and 29.10 Mb, clearly suggesting the presence of two resistance loci (Fig. 3). Previously Zhan et al. (2008) suggested that the name *Rrs17* should be assigned to $Rrs15_{CIho8288}$ as it was found at a different locus to *Rrs15* on 7H (Dahleen 2006; Zhan et al. 2008). Therefore, we suggest that the resistance locus identified as a QTL on 6HS in SxM and CxA populations should be designated *Rrs18*.

The QTL on 6H in Post x Vixen population was identified using isolate 271 (Wagner et al. 2008), the same isolate as was used for fine mapping with SxM BC_1 lines. Flanking markers for a QTL effect previously identified in 3 cultivars Keele, Harrington and O'Connor (Cheong et al. 2006) can also be located to the same physical position as the *Rrs18* locus. The QTL identified by Cheong et al. (2006) was found using natural inoculum, and the position of the QTL peak (at marker ABG378), strongly suggests that the QTL effect represents an allele of *Rrs18*. A single major resistance gene identified in a Vlamingh x WABAR2147 DH population by Wang et al. (2014) on 6HS has flanking markers 1_1166 and Bmag500, located at 7.47 Mb and 16.14 Mb respectively, putting this QTL also in the vicinity of *Rrs18*. The resistance gene on 6HS in WABAR2147 was shown to be effective irrespective of growth stage, so if the conclusion that this resistance is an allele of *Rrs18* is correct, it would suggest that *Rrs18* would provide an effective resistance in the field (Wang et al. 2014).

Another highly significant QTL to *R. commune* isolate UK7 identified using SxM DH population on 3H, qSUK7_3, which explained 30.4 % of the total phenotypic variation, with Steptoe providing the resistant allele, is in a similar physical position to that of $Rrs1_{Rh4}$ (Table 2). However, Rhy17 which is recognised by

$Rrs1_{Rh4}$ is virulent on Steptoe (Table 1), suggesting strongly that $Rrs1_{Rh4}$ is not present in Steptoe. Multiple rhynchosporium resistance QTL have been identified on 3H using different isolates and different backgrounds (Zhan et al. 2008), though the race specific nature of qSUK7_3 suggests it could be another major resistance gene. QTL qSUK7_3 was mapped to a large interval overlapping with the *Rrs1* region identified in the CxA population (Fig. 1d, Fig. 2) but quite far from *Rrs4*, which is located closer to the telomere (Patil et al. 2003, Zhan et al. 2008).

The fine mapping of *Rrs18* with *R. commune* isolate L73a was confirmed using isolate 271, which was used for the original SxM DH QTL mapping (Fig. 1a, Fig. 4b). Independent mapping with each of these isolates resulted in similar physical intervals of 9.19-13.88 Mb for isolate L73a, and 9.08-11.78 Mb for isolate 271. Further fine mapping using L73a narrowed down the *Rrs18* interval to 660 kb, 10.92-11.58 Mb (Fig. 4c).

Given the similar map positions, and the fact that each population shows the same response to specific isolates, it is reasonable to assume that the resistance QTL on 6HS in SxM and CxA populations contains the same resistance gene. Therefore, the gene causing resistance in Steptoe should have some allelic similarity to the gene in CIho 3515 with alternative allele(s) in Morex and Alexis. Obviously the *Rrs18* gene should be expressed in Steptoe and CIho 3515. All 4 transcribed genes within *Rrs18* region showed sequence variants between CIho 3515 and Alexis, though only 3 of them, including a putative protein kinase, a putative allene oxide synthase and a putative elongation factor P, had SNPs specific to Steptoe and CIho 3515, compared to Morex and Alexis (Table 5). Furthermore, only 2 of these SNPs could lead to a non-synonymous substitution: one SNP in putative allene oxide synthase HORVU6Hr1G005250, and one SNP in putative protein kinase HORVU6Hr1G005260 (Table 5).

Allene oxide synthase is the first enzyme involved in the so-called LOX pathway leading to synthesis of the plant hormone jasmonic acid (JA) (Maucher et al., 2000). JA is generally associated with regulation of defence genes, in particular - against nectrotrophic pathogens and insects, while salicylic acid (SA), another plant hormone, is associated with regulating genes involved in defence against biotrophic pathogens (Glazebrook, 2005). Despite its association with plant defence against pathogens, as an enzyme and not a receptor, allene oxide synthase is an unlikely candidate for an *R* gene.

Like many other agronomically important pathogens including, *Zymoseptoria tritici*, *Mycosphaerella fijiensis*, *Cladosporium fulvum* and *Leptosphaeria maculans*, causing major diseases affecting wheat, banana, tomato and oilseed rape, respectively, *R. commune* colonises the plant extracellular space (Jones and Ayres 1974; Lehnackers and Knogge 1990; Thirugnanasambandam et al. 2011; Stotz et al. 2014). Therefore, its effectors, or their effect on the plant, are likely to be recognised at the plant cell surface by *R* genes encoding cell surface-localised receptor-like kinases (RLKs) or receptor-like proteins (RLPs) (Avrova and Knogge 2012; Stotz et al. 2014, Saintenac et al. 2018). Three tomato *R* genes against *C. fulvum*, *Cf-2*, *Cf-4* and *Cf-9*, are RLPs shown to interact with the RLK SOBIR1/EVR for downstream signalling and defence (Liebrand, et al. 2013). Oilseed rape resistance against *L. maculans* and apple (*Malus domestica*) resistance against *V. inaequalis* also involve RLPs (Larkan et al. 2013; Vinatzer et al. 2001; Xu and Korban 2002). Recently the wheat receptor kinase-like protein Stb6 has been shown to control gene-for-gene resistance to *Z. tritici* (Saintenac et al. 2018). Similar to *Rrs18*, *Stb6* confers pathogen resistance in the absence of a hypersensitive response (Saintenac et al. 2018).

Our results showed *Rrs18* to be dominant, making the putative protein kinase HORVU6Hr1G005260 the most likely candidate. The predicted protein sequence of HORVU6Hr1G005260 contains a potential extracellular domain with a signal peptide, a transmembrane domain and a serine/threonine kinase domain. It has one SNP matching the expected allele segregation leading to a non-synonymous substitution in the potential extracellular receptor domain (Table 5). As the amino acid substitution is neutral and has no deleterious effect on the protein due to its position outside the catalytic domain, it fits with the hypothesis that this particular protein kinase evolved to recognise the presence of *R. commune*. Similar to HORVU6Hr1G005260, the susceptible haplotype of *Stb6*, differs from the resistant haplotype by a single nonsynonymous SNP, in this case in the S/T kinase domain (Saintenac et al. 2018). Further tests are needed to find out whether any of the identified SNPs correlate with the presence/absence of *Rrs18* in other genotypes.

HORVU6Hr1G005260 is highly similar (94 % identity) to a cysteine-rich receptor kinase NCRK from *Aegilops tauschii* subsp. *tauschii.* NCRK from Arabidopsis was shown to interact with Rop GTPase at the plant plasma membrane (Molendijk et al. 2008). In plants, Rop GTPases are central regulators of diverse signalling pathways in plant growth and, most importantly in this case, pathogen defence.

Another possibility is that *Rrs18* is not present in susceptible Morex and further sequencing of RNA from the resistant lines, containing *Rrs18* and *de novo* assembly might reveal additional candidate gene(s). In addition, sufficiently replicated RNA-seq analysis would allow assessment of whether any of the genes within the *Rrs18* interval are differentially expressed between the resistant and susceptible parents. Ultimately, transformation of susceptible barley cultivar Golden Promise with the resistant allele of the identified candidate gene(s) for *Rrs18* is needed to confirm its function.

Author contribution statement

MC, KH, GS, MEL and AA designed the experiments. MC, BB, KH and MEL performed the experiments. MC, KH, MB, MEL and AA analysed the data. MC, MEL and AA wrote the manuscript with input from BB, MB, LR, GS and RW.

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Conflict of interest

The authors declare that they have no conflict of interest.

References

Abbott DC, Burdon JJ, Jarosz AM, Brown AHD, Muller WJ, Read BJ (1991) The relationship between seedling infection types and field reactions in Clipper barley backcross lines. Aust J Agric Res 42:801-809

Abbott DC, Lagudah ES, Brown AHD (1995) Identification of RFLPs flanking a scald resistance gene on barley chromosome 6. J Heredity 86:152-154

Abramoff MD, Magalhães PJ, Ram SJ (2004) Image Processing with ImageJ. Biophotonics Internat 11:36-42

Avrova A, Knogge W (2012) *Rhynchosporium commune*: a persistent threat to barley cultivation. Mol Plant Pathol 13:986-997

Bates D, Maechler M, Bolker B, Walker S (2015) Fitting Linear Mixed-Effects Models Using lme4. J Statistical Software 67:1-48

Bayer MM, Rapazote-Flores P, Ganal M, Hedley PE, Macaulay M, Plieske J, Ramsay L, Russell J, Shaw PD, Thomas W, Waugh R (2017) Development and Evaluation of a Barley 50k iSelect SNP Array. Front Plant Sci doi: 10.3389/fpls.2017.01792

Behn A, Hartl L, Schweizer G, Wenzel G, Baumer M (2004) QTL mapping for resistance against non-parasitic leaf spots in a spring barley doubled haploid population. Theor Appl Genet 108:1229-1235

Bjørnstad A, Patil V, Tekauz A, Maroy AG, Skinnes H, Jensen A, Magnus H, MacKey J (2002) Resistance to scald (*Rhynchosporium secalis*) in barley (*Hordeum vulgare*) studied by near-isogenic lines: I. Markers and differential isolates. Phytopathol 92:710-720

Broman KW, Wu H, Sen S, Churchill GA (2003) R/qtl: QTL mapping in experimental crosses. Bioinformatics (Oxford, England) 19:889-890

Cheong J, Williams K, Wallwork H (2006) The identification of QTLs for adult plant resistance to leaf scald in barley. Aust J Agric Res 57:961-965

Cingolani P, Platts A, Wang LL, Coon M, Tung N, Wang L, Land SJ, Lu X, Ruden DM (2012) A program for annotating and predicting the effects of single nucleotide polymorphisms, SnpEff: SNPs in the genome of *Drosophila melanogaster* strain w(1118); iso-2; iso-3. *Fly* 6:80-92. doi: 10.4161/fly.19695

Close TJ, Bhat PR, Lonardi S, Wu Y, Rostoks N, Ramsay L, Druka A, Stein N, Svensson J, Wanamaker S, Bozdag S, Roose M, Moscou M, Chao S, Varshney R, Szucs P, Sato K, Hayes P, Matthews D, Kleinhofs A, Muehlbauer G, DeYoung J, Marshall D, Madishetty K, Fenton R, Condamine P, Graner A, Waugh R (2009) Development and implementation of high-throughput SNP genotyping in barley. BMC Genomics 10:582-594

Dahleen LS (2006) Coordinator's report: chromosome 7H. Barley Genetics Newsletter 36:63-65

Davis H, Fitt BD (1990) Symptomless infection of *Rhynchosporium secalis* on leaves of winter barley. Mycol Res 94:557-560

Dobin A, Davis CA, Schlesinger F, Drenkow J, Zaleski C, Jha S, Batut P, Chaisson M and Gingeras TR (2013) STAR: ultrafast universal RNA-seq aligner. Bioinformatics 29:15-21. doi: 10.1093/bioinformatics/bts635

Druka A, Druka I, Centeno AG, Li H, Sun Z, Thomas WTB, Bonar N, Steffenson BJ, Ullrich SE, Kleinhofs A, Wise RP, Close TJ, Potokina E, Luo Z, Wagner C, Schweizer GF, Marshall DF, Kearsey MJ, Williams RW, Waugh R (2008) Towards systems genetic analyses in barley: Integration of phenotypic, expression and genotype data into GeneNetwork. BMC Genet 9:73-83

Genger RK, Brown AHD, Knogge W, Nesbitt K, Burdon JJ (2003) Development of SCAR markers linked to a scald resistance gene derived from wild barley. Euphytica 134:149-159

Glazebrook J (2005) Contrasting mechanisms of defense against biotrophic and necrotrophic pathogens. Ann Rev Phytopathol 43:205-227

Habgood MR, Hayes JD (1971) The inheritance of resistance to *Rhynchosporium secalis* in barley. Heredity 27:25–37

Hanemann A, Schweizer GF, Cossu R, Wicker T, Röder MS (2009) Fine mapping, physical mapping and development of diagnostic markers for the *Rrs2* scald resistance gene in barley. Theor Appl Genet 119:1507-1522

Hofmann K, Silvar C, Casas AM, Herz M, Büttner B, Gracia MP, Contreras-Moreira B, Wallwork H, Igartua E, Schweizer G (2013) Fine mapping of the *Rrs1* resistance locus against scald in two large populations derived from Spanish barley landraces. Theor Appl Genet 126:3091-3102

IBSC International Barley Genome Sequencing Consortium (2012) A physical, genetic and functional sequence assembly of the barley genome. Nature 491:711-716

Jackson LF, Webster RK (1976) Race differentiation, distribution, and frequency of *Rhynchosporium secalis* in California. Phytopathol 66:719-725

Jenkins JEE, Jemmett JL (1967) Barley leaf blotch. NAAS Quarterly Review 75:127-132

Jensen J, Backes G, Skinnes H, Giese H (2002) Quantitative trait loci for scald resistance in barley localized by a non-interval mapping procedure. Plant Breed 121:124-128

Jones P, Ayres PG (1974) Rhynchosporium leaf blotch of barley studied during the subcuticular phase by electron microscopy. Physiol Plant Pathol 4:229-233

Kleinhofs A, Kilian A, Saghai Maroof MA, Biyashev RM, Hayes P, Chen FQ, Lapitan N, Fenwick A, Blake TK, Kanazin V, Ananiev E, Dahleen L, Kudrna D, Bollinger J, Knapp SJ, Liu B, Sorrells M, Heun M, Franckowiak JD, Hoffman D, Skadsen R, Steffenson BJ (1993) A molecular, isozyme and morphological map of the barley (*Hordeum vulgare*) genome. Theor Appl Genet 86:705-712

Kuznetsova A, Brockhoff PB, Christensen RHB (2017) lmerTest Package: Tests in Linear Mixed Effects Models. J Statistical Software 82:1-26

Larkan NJ, Lydiate DJ, Parkin IAP, Nelson MN, Epp DJ, Cowling WA, Rimmer SR, Borhan MH (2013) The *Brassica napus* blackleg resistance gene *LepR3* encodes a receptor-like protein triggered by the *Leptosphaeria maculans* effector AVRLM1. New Phytol 197:595-605

Lehnackers H, Knogge W (1990) Cytological studies on the infection of barley cultivars with known resistance genotypes by Rhynchosporium secalis. Can J Bot 68:1953-1961

Li J, Ji L (2005). Adjusting multiple testing in multilocus analyses using the eigenvalues of a correlation matrix. Heredity 95:221-227

Li JZ, Sjakste TG, Röder MS, Ganal MW (2003) Development and genetic mapping of 127 new microsatellite markers in barley. Theor Appl Genet 107:1021-1927

Li H, Durbin R (2009) Fast and accurate short read alignment with Burrows-Wheeler transform. Bioinformatics 25**:**1754-1760

Liebrand TWH, van den Berg GCM, Zhang Z, Smit P, Cordewener JHG, America AHP, Sklenar J, Jones AME, Tameling WIL, Robatzek S, Thomma BPHJ, Joosten MHAJ (2013) Receptor-like kinase SOBIR1/EVR interacts with receptor-like proteins in plant immunity against fungal infection. Proc Natl Acad Sci USA 110:10010-10015

Looseley ME, Griffe LL, Büttner B, Wright KM, Middlefell-Williams J, Bull H, Shaw PD, Macaulay M, Booth A, Schweizer G, Russell JR, Waugh R, Thomas WTB, Avrova A (2018) Resistance to *Rhynchosporium commune* in a collection of European spring barley germplasm. Theor Appl Genet doi: 10.1007/s00122-018-3168-5

Marzin S, Hanemann A, Sharma S, Hensel G, Kumlehn J, Schweizer G, Röder MS (2016) Are PECTIN ESTERASE INHIBITOR Genes Involved in Mediating Resistance to *Rhynchosporium commune* in Barley? PLoS ONE 11:e0150485

Mascher M, Richmond TA, Stein N (2013) Barley whole exome capture: a tool for genomic research in the genus Hordeum and beyond. Plant J 76:494-505

Mascher M, Gundlach H, Himmelbach A, Beier S, Twardziok SO, Wicker T, Radchuk V, Dockter C, Hedley PE, Russell J, Bayer M, Ramsay L, Liu H, Haberer G, Zhang X-Q, Zhang Q, Barrero RA, Li L, Taudien S, Groth M, Felder M, Hastie A, Šimková H, Staňková H, Vrána J, Chan S, Muñoz-Amatriaín M, Ounit R, Wanamaker S, Bolser D, Colmsee C, Schmutzer T, Aliyeva-Schnorr L, Grasso S, Tanskanen J, Chailyan A, Sampath D, Heavens D, Clissold L, Cao S, Chapman B, Dai F, Han Y, Li H, Li X, Lin C, McCooke JK, Tan C, Wang P, Wang S, Yin S, Zhou G, Poland JA, Bellgard MI, Borisjuk L, Houben A, Doležel J, Ayling S, Lonardi S, Kersey P, Langridge P, Muehlbauer GJ, Clark MD, Caccamo M, Schulman AH, Mayer KFX, Platzer M, Close TJ, Scholz U, Hansson M, Zhang G, Braumann I, Spannagl M, Li C, Waugh R, Stein N (2017) A chromosome conformation capture ordered sequence of the barley genome. Nature 544:427-443

Maucher H, Hause B, Feussner I, Ziegler J, Wasternack C (2000) Allene oxide synthases of barley (Hordeum vulgare cv. Salome): tissue specific regulation in seedling development. Plant J 21:199-213

Mayer KFX, Martis M, Hedley PE, Šimková H, Liu H, Morris JA, Steuernagel B, Taudien S, Roessner S, Gundlach H, Kubaláková M, Suchánková P, Murat F, Felder M, Nussbaumer T, Graner A, Salse J, Endo T, Sakai H, Tanaka T, Itoh T, Sato K, Platzer M, Matsumoto T, Scholz U, Doležel J, Waugh R, Stein N (2011) Unlocking the barley genome by chromosomal and comparative genomics. Plant Cell 23:1249-1263

Mckenna A, Hanna M, Banks E, Sivachenko A, Cibulskis K, Kernytsky A, Garimella K, Altshuler D, Gabriel S, Daly M, Depristo MA (2010) The Genome Analysis Toolkit: A MapReduce framework for analyzing next-generation DNA sequencing data. Genome Res 20**:**1297-1303

Milne I, Bayer M, Cardle L, Shaw P, Stephen G, Wright F, Marshall D (2010a) Tablet - next generation sequence assembly visualization. Bioinformatics 26:401-402

Milne I, Shaw P, Marshall D (2010b) Flapjack - graphical genotype visualization. Bioinformatics 26:3133-3134

Milne I, Stephen G, Bayer M, Cock PJA, Pritchard L, Cardle L, Shaw PD, Marshall D (2013) Using Tablet for visual exploration of second-generation sequencing data. Briefings in Bioinformatics 14**:**193-202

Molendijk AJ, Ruperti B, Singh MK, Dovzhenko A, Ditengou FA, Milia M, Westphal L, Rosahl S, Soellick T-R, Uhrig J, Weingarten L, Huber M, Palme K (2008) A cysteine-rich receptor-like kinase NCRK and a pathogeninduced protein kinase RBK1 are Rop GTPase interactors. Plant J 53: 909-923

Newton AC (1989) Somatic recombination in *Rhynchosporium secalis*. Plant Pathol 38:71-74

Newton AC, Searle J, Guy DC, Hackett CA, Cooke DEL (2001) Variability in pathotype, aggressiveness, RAPD profile, and rDNA ITS1 sequences of UK isolates of *Rhynchosporium secalis*. Zeitschrift fur Pflanzenkrankheiten und Pflanzenschutz-Journal of Plant Diseases and Protection 108:446-458

Oxley SJP, Cooke LR, Black L, Hunter A, Mercer PC *(*2003) Management of Rhynchosporium in Different Barley Varieties and Cropping Systems. London, UK: Home-Grown Cereals Authority, Project Report 315.

Patil V, Bjørnstad Å, Mackey J (2003) Molecular mapping of a new gene $Rrs4_{CI\ 11549}$ for resistance to barley scald (*Rhynchosporium secalis*) Mol Breeding 12:169-183

Phillips D, Jenkins G, Ramsay L (2015) The effect of temperature on the male and female recombination landscape of barley. New Phytol 208:241-249

R Core Team (2012) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, URL http://www.R-project.org/

Ramsay L, Macaulay M, Ivanissevich SD, MacLean K, Cardle L, Fuller J, Edwards KJ, Tuvesson S, Morgante M, Massari A, Maestri E, Marmiroli N, Sjakste T, Ganal M, Powell W, Waugh R (2000) A Simple Sequence Repeat-Based Linkage Map of Barley. Genetics 156:1997-2005

Ribeiro A, Golicz A, Hackett CA, Milne I, Stephen G, Marshall D, Flavell AJ, Bayer M (2015) An investigation of causes of false positive single nucleotide polymorphisms using simulated reads from a small eukaryote genome. BMC Bioinformatics 16**:**382-397

Rohe M, Searle J, Newton AC, Knogge W (1996) Transformation of the plant pathogenic fungus, *Rhynchosporium secalis*. Curr Genet 29:587-590

Rostoks N, Mudie S. Cardle L, Russell J, Ramsay L, Booth A, Svensson JT, Wanamaker SI, Walia H, Rodriguez EM, Hedley PE, Liu H, Morris J, Close TJ, Marshall DF, Waugh R (2005) Genome-wide SNP discovery and linkage analysis in barley based on genes responsive to abiotic stress Mol Genet Genomics 274:515-527

Russell J, Mascher M, Dawson IK, Kyriakidis S, Calixto C, Freund F, Bayer M, Milne I, Marshall-Griffiths T, Heinen S, Hofstad A, Sharma R, Himmelbach A, Knauft M, Van Zonneveld M, Brown JWS, Schmid K, Kilian B, Muehlbauer GJ, Stein N, and Waugh R (2016) Exome sequencing of geographically diverse barley landraces and wild relatives gives insights into environmental adaptation. Nat Genet 48:1024-1030. doi: 10.1038/ng.3612

Saintenac C, Lee WS, Cambon F, Rudd JJ, King RC, Marande W, Powers SJ, Bergès H, Phillips AL, Uauy C, Hammond-Kosack KE, Langin T, Kanyuka K (2018) Wheat receptor kinase-like protein Stb6 controls gene-for-gene resistance to fungal pathogen *Zymoseptoria tritici*. Nat Genet doi: 10.1038/s41588-018-0051-x.

Schweizer GF, Baumer M, Daniel G, Rugel H, Röder MS (1995) RFLP markers linked to scald (*Rhynchosporium secalis*) resistance gene Rh2 in barley. Theor Appl Genet 90:920-924

Shipton WA, Boyd WJR and Ali SM (1974) Scald of barley. Rev Plant Pathol 53:839-861

Shtaya MJY, Marcel TC, Sillero JC, Niks RE, Rubiales D (2006) Identification of QTLs for powdery mildew and scald resistance in barley. Euphytica 151:421-429

Silvar C, Casas AM, Igartua E, Ponce-Molina LJ, Gracia MP, Schweizer G, Herz M, Flath K, Waugh R, Kopahnke D, Ordon F (2011) Resistance to powdery mildew in Spanish barley landraces is controlled by different sets of quantitative trait loci. Theor Appl Genet 123:1019-1028

Starling TM, Roane CW, Chi KR (1971) Inheritance of reaction to *Rhynchosporium secalis* in winter barley cultivars. In: Proceedings of 2nd Int Barley Genetics Symposium, Pullman, WA, 513-519

Stein N, Prasad M, Scholz U, Thiel T, Zhang H, Wolf M, Kota R, Varshney RK, Perovic D, Grosse I, Graner A (2007) A 1,000-loci transcript map of the barley genome: new anchoring points for integrative grass genomics. Theor Appl Genet 114:823-839

Stotz H, Mitrousia G, de Wit P, Fitt BDL (2014) Effector-triggered defence against apoplastic fungal pathogens. Trends Plant Sci 19:491-500. doi: 10.1016/j.tplants.2014.04.009

Thiel T, Kota R, Grosse I, Stein N, Graner A (2004) SNP2CAPS: a SNP and INDEL analysis tool for CAPS marker development, Nucleic Acids Res 32:e5

Thirugnanasambandam A, Wright KM, Atkins SD, Whisson SC, Newton AC (2011) Infection of Rrs1 barley by an incompatible race of the fungus Rhynchosporium secalis expressing the green fluorescent protein. Plant Pathol 60:513-521

Van Der Auwera GA, Carneiro MO, Hartl C, Poplin R, Del Angel G, Levy-Moonshine A, Jordan T, Shakir K, Roazen D, Thibault J, Banks E, Garimella KV, Altshuler D, Gabriel S, Depristo MA (2013) From FastQ data to high confidence variant calls: the Genome Analysis Toolkit best practices pipeline. Current protocols in Bioinformatics 43**:**11-33

Varshney RK, Mahendar T, Aggarwal RK, Börner A. (2007) Genic Molecular Markers in Plants: Development and Applications. In: Varshney R.K., Tuberosa R. (eds) Genomics-Assisted Crop Improvement. Springer, Dordrecht

Vinatzer BA, Patocchi A, Gianfranceschi L, Tartarini S, Zhang HB, Gessler C, Sansavini S (2001) Apple contains receptor-like genes homologous to the *Cladosporium fulvum* resistance gene family of tomato with a cluster of genes cosegregating with Vf apple scab resistance. Mol Plant Microbe Interact 14:508-515

VSN International (2014) GenStat for Windows 17th edn. VSN International, Hemel Hempstead, UK

Wagner C, Schweizer G, Krämer M, Dehmer-Badani AG, Ordon F, Friedt W (2008) The complex quantitative barley-*Rhynchosporium secalis* interaction: newly identified QTL may represent already known resistance genes. Theor Appl Genet 118:113-122

Wang Y, Gupta S, Wallwork H, Zhang X-Q, Zhou G, Broughton S, Loughman R, Lance R, Wu D, Shu X, Li C (2014) Combination of seedling and adult plant resistance to leaf scald for stable resistance in barley. Mol Breed 34:2081-2089

Xi K, Burnett PA, Tewari JP, Chen MH, Turkington TK, Helm JH (2000) Histopathological study of barley cultivars resistant and susceptible to *Rhynchosporium secalis*. Phytopathol 90:94-102

Xu M, Korban SS (2002) A cluster of four receptor-like genes resides in the Vf locus that confers resistance to apple scab disease. Genetics 162:1995-2006

Zhan J, Fitt BDL, Pinnschmidt HO, Oxley SJP, Newton AC (2008) Resistance, epidemiology and sustainable management of *Rhynchosporium secalis* populations on barley. Plant Pathol 57:1-14

Table 1. Disease reactions of 8 barley cultivars and landraces against 9 *Rhynchosporium commune* isolates

Barley lines	*R. commune* isolates									R-gene(s) according to literature
	Disease scores on a 1-4 scale following spray inoculation								Mean lesion size, mm	
	271	UK7	R214	Rhy174	S147-1	LfL07	SGü4/3	Rhy17	L73a	
Steptoe	0.9 (0.1)[a]	0.0 (0.0)	0.1 (0.2)	0.3 (0.1)	0.6 (0.4)	1.3 (1.2)	2.0 (0.4)	4.0 (0.0)	10.1 (1.1)	?
CIho 3515	0.1 (0.1)	0.3 (0.0)	0.0 (0.0)	0.3 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	8.0 (1.2)	*Rrs1*$_{Rh4}$ + *Rrs13* (Hofmann et al. 2013)
SBCC145	0.0 (0.0)	0.3 (0.0)	0.0 (0.0)	0.2 (0.1)	0.0 (0.0)	0.1 (0.1)	0.0 (0.0)	0.0 (0.0)		*Rrs1*$_{Rh4}$ (Hofmann et al. 2013)
Atlas	1.0 (0.0)	1.2 (0.3)	3.3 (1.3)	4.0 (0.0)	3.8 (0.3)	4.0 (0.0)	4.0 (0.0)	4.0 (0.0)		*Rrs2* (Hanemann et al. 2009)
Morex	2.8 (0.5)	3.8 (0.3)	1.3 (0.0)	4.0 (0.0)	2.8 (0.4)	4.0 (0.0)	4.0 (0.0)	4.0 (0.0)	14.8 (1.1)	susceptible
Alexis	4.0 (0.0)	4.0 (0.0)	3.3 (1.3)	4.0 (0.0)	3.3 (0.6)	4.0 (0.0)	4.0 (0.0)	4.0 (0.0)	14.2 (0.9)	susceptible
Beatrix	4.0 (0.0)	4.0 (0.0)	4.0 (0.0)	3.8 (0.3)	4.0 (0.0)	4.0 (0.0)	4.0 (0.0)	4.0 (0.0)		susceptible
Steffi	4.0 (0.0)	2.9 (1.3)	3.1 (1.1)	4.0 (0.0)	4.0 (0.0)	4.0 (0.0)	4.0 (0.0)	4.0 (0.0)		susceptible

[a] Standard error

Table 2. QTL for rhynchosporium resistance identified using Steptoe x Morex and CIho 3515 x Alexis DH populations and *Rhynchosporium commune* isolates 271, UK7, LfL07, S147-1and Rhy174

Population	*R. commune* isolate	QTL Chromosome	QTL name	QTL Flanking markers	QTL interval, cM	QTL interval, Mb	Resistant parent allele	-log10(p)	R^2, %	Additive effect
Steptoe x Morex	271	3H	qS271_3	2_0023, 1_0253	93.2 - 111.4	591.89 – 617.76	Morex	7.1	12.6	0.19
		6H	qS271_6a	2_0232, 1_0023	0 – 24.4	1.57 – 16.13	Steptoe	16.3	30.1	0.30
		6H	qS271_6b	1_0129, 1_1475	51.6 - 67.3	30.80 – 463.86	Morex	5.9	9.6	0.17
		7H	qS271_7	2_1448, 1_0885	81.7 - 118.2	576.34 – 638.91	Steptoe	4.3	7.4	0.15
	UK7	3H	qSUK7_3	1_1342, 2_1129	61.9 - 66.6	201.16 – 508.77	Steptoe	24.5	30.4	0.65
		5H	qSUK7_5	1_1135, 2_0265	113.0 - 135.7	456.06 – 525.97	Steptoe	7.2	6.9	0.31
		6H	qSUK7_6	2_0262, 1_1479	9.4 - 16.6	7.89 – 12.01	Steptoe	34.8	41.0	0.75
CIho 3515 x Alexis	LfL07	3H	qC07_3	GBM1094, STSagtc17	35.7 - 45.2	457.98 – 542.28	CIho 3515	90.6	63.7	1.07
		6H	qC07_6	U35_24165, GBS0346	6.3 - 18.9	10.01 – 14.33	CIho 3515	11.8	6.3	0.34
	S147-1	3H	qC147_3	GBM1094, Bmag0112	35.7 – 50.0	457.98 – 542.28	CIho 3515	74.5	59.5	1.07
		6H	qC147_6	U35_24165, GBS0346	6.3 - 18.9	10.01 – 14.33	CIho 3515	15.6	11.7	0.48
	Rhy174	3H	qC174_3	GBM1094, GMS0116	35.7 - 57.7	457.98 – 557.36	CIho 3515	6.0	3.9	0.27
		6H	qC174_6	U35_24165, U35_40281	6.3 - 17.2	10.01 – 12.05	CIho 3515	97.1	68.9	1.14

R^2 is the percentage of phenotypic variance explained by the QTL

Table 3. Comparison of REML predicted means for *Rrs18* genotypes

Differences between average greatest lesion size, mm		
	Steptoe	Morex
Morex	2.6**	-
Heterozygous	0.5	2.0*

*Significant at $P < 0.05$

**Significant at $P < 0.01$

Table 4. Genes annotated within *Rrs18* interval in Morex genome assembly v4

Gene ID	Start position, bp	End position, bp	Putative function	Gene annotation confidence
HORVU6Hr1G005080*	10,921,339	10,925,628	Elongation factor P-like protein, putative isoform 2	high
HORVU6Hr1G005100	10,927,482	10,931,218	Ubiquitin carboxyl-terminal hydrolase family protein	high
HORVU6Hr1G005110	11,067,497	11,069,706	Transposon Ty1-OL Gag-Pol polyprotein	low
HORVU6Hr1G005120	11,263,448	11,264,902	F-box domain containing protein	high
HORVU6Hr1G005130	11,277,874	11,278,486	undescribed protein	low
HORVU6Hr1G005140	11,280,514	11,281,715	undescribed protein	high
HORVU6Hr1G005150	11,288,602	11,298,978	two-component response regulator ARR11	high
HORVU6Hr1G005170	11,314,367	11,326,412	two-component response regulator ARR11	high
HORVU6Hr1G005190	11,391,525	11,392,142	Transposon protein, putative, Mutator sub-class	low
HORVU6Hr1G005200	11,400,174	11,400,372	undescribed secreted protein	low
HORVU6Hr1G005210	11,441,330	11,442,077	undescribed protein	low
HORVU6Hr1G005220	11,475,836	11,480,126	60 kDa chaperonin 2	high
HORVU6Hr1G005230	11,493,928	11,495,397	Membrane fusion protein Use1	high
HORVU6Hr1G005240*	11,509,347	11,515,094	Pentatricopeptide repeat-containing protein	high
HORVU6Hr1G005250*	11,516,051	11,518,503	Allene oxide synthase	high
HORVU6Hr1G005260*	11,567,133	11,574,185	Protein kinase superfamily protein	high
HORVU6Hr1G005280	11,574,189	11,574,510	undescribed protein	low

* Transcribed in CIho 3515 and Alexis leaves

Table 5. Genotypes for lines CIho3515, Steptoe, Morex and Alexis within *Rrs18* interval based on Flapjack genotype visualization software (Milne et al. 2010b). Lines were sorted by similarity to Alexis with Alexis allele highlighted in grey.

Gene ID	SNP	Barley line				SNP effect
		Morex	Alexis	CIho3515	Steptoe	
HORVU6Hr1G005080	chr6H_10922107	G	A	G	G	P/S
	chr6H_10924478	C	C	T	T	Synonymous
HORVU6Hr1G005240	chr6H_11509890	C	T	C	C	Syn
	chr6H_11509979	T	T	C	T	V/A
	chr6H_11510387	C	C	C/T	C	P/L
HORVU6Hr1G005250	chr6H_11516570	A	A	G	G	3'UTR
	chr6H_11517174	G	T	G	G	Synonymous
	chr6H_11517367	G	A	G	G	Synonymous
	chr6H_11517718	C	C	A	C	Synonymous
	chr6H_11517940	G	G	A	A	Synonymous
	chr6H_11518293	G	G	C	C	L/V
	chr6H_11518315	T	T	C	C	5'UTR
HORVU6Hr1G005260	chr6H_11571800	A	A	G	G	T/A
	chr6H_11572699	T	C	T	T	Synonymous
	chr6H_11572843	A	C	A	C	Intron
	chr6H_11572964	T	C	T	T	Synonymous
	chr6H_11573501	G	A	G	A	Synonymous
	chr6H_11573820	A	G	A	A	S/G
	chr6H_11574036	A	A	C	A	3'UTR

Figure legends

Fig. 1 Response of 140 Steptoe x Morex DH lines to *Rhynchosporium commune* isolates **a** 271 and **b** UK7. The Jackson and Webster (1976) scale extended by half steps was used. Vertical arrows indicate disease scores of parental cultivars Steptoe and Morex, and mean disease scores. A single environment QTL scan for disease severity using *R. commune* isolates **c** 271 and **d** UK7 on Steptoe x Morex DH population. The horizontal dashed line represents the significance threshold corresponding to a genome-wide error rate of 0.05.

Fig. 2 Response of 238, 239 and 238 CIho 3515 x Alexis DH lines to *Rhynchosporium commune* isolates **a** LfL07, **b** S147-1and **c** Rhy174 respectively. The Jackson and Webster (1976) scale extended by half steps was used. Vertical arrows indicate disease scores of parents CIho 3515 and Alexis, and mean disease scores. **d** QTL on chromosomes 3H and 6H of CIho 3515 x Alexis DH population for *R. commune* isolates LfL07, S147-1 and Rhy174. The horizontal dashed line represents the significance threshold corresponding to an overall error rate of 0.05.

Fig. 3 Positions of marker sequences on 6HS pseudomolecule of Morex genome assembly v4. The diagram shows the first 30 Mb of the Morex 6HS pseudomolecule. Brackets show the flanking markers for QTL identified for resistance to rhynchosporium, and the parental lines used in QTL mapping. The physical position of *Rrs13* was identified using flanking markers Cxp3 and MWG916 from BC line 30 x Clipper population (Genger et al. 2003) and flanking markers BMag500 and MWG916 from AB30 x Clipper BC_3F_2 population (Cheong et al. 2006). The resistance QTL identified in Steptoe x Morex and CIho 3515 x Alexis DH populations at roughly the same position distal to *Rrs13* have been assigned as the *Rrs18* locus.

Fig. 4 Fine mapping of rhynchosporium severity on selected Steptoe x Morex BC_1S_2 lines using *Rhynchosporium commune* isolates **a** 271, **b** L73a. Further fine mapping was carried out with additional Steptoe x Morex BC_1S_2 lines using *R. commune* isolate L73a (**c**). The horizontal dashed line shows the Logarithm of the odds (LOD) corresponding to an overall error rate of 0.05.

Fig. 5 Partial genetic map of chromosome 6H, linked to physical map, depicting the markers used for fine mapping of *Rrs18* in Steptoe x Morex BC_1 population, and gene content within *Rrs18* interval based on the latest Morex genome annotation (Mascher et al. 2017) **(**not drawn to scale). Black rectangles represent genes transcribed in leaves of CIho3515 and Alexis, dark and light grey rectangles represent remaining high and low confidence genes respectively.

Supplementary Tables

Table S1 Genotypes and mean disease scores of Steptoe x Morex DH lines screened with *Rhynchosporium commune* isolates 271 and UK7

Table S2 Genotypes and mean disease scores of CIho 3515 x Alexis DH lines screened with *Rhynchosporium commune* isolates LfL07, S147-1 and Rhy174

Table S3 Markers used for Steptoe x Morex DH population mapping with genetic map positions

Table S4 Markers used for CIho 3515 x Alexis DH population mapping with genetic map positions

Table S5 OPA markers used for selecting Steptoe x Morex BC_1 S_1 lines for mapping

Table S6 KASP markers with primer sequences

Table S7 BeadXpress OPA markers used for genotyping Steptoe x Morex BC_1S_2 lines for mapping

Table S8 Genotypes and phenotypes of Steptoe x Morex BC_1S_2 lines used in fine mapping

Table S9 Steptoe x Morex BC_1S_2 lines genotypes and average greatest lesion size following inoculation with *Rhynchosporium commune* isolate L73a, used in the final fine mapping experiment

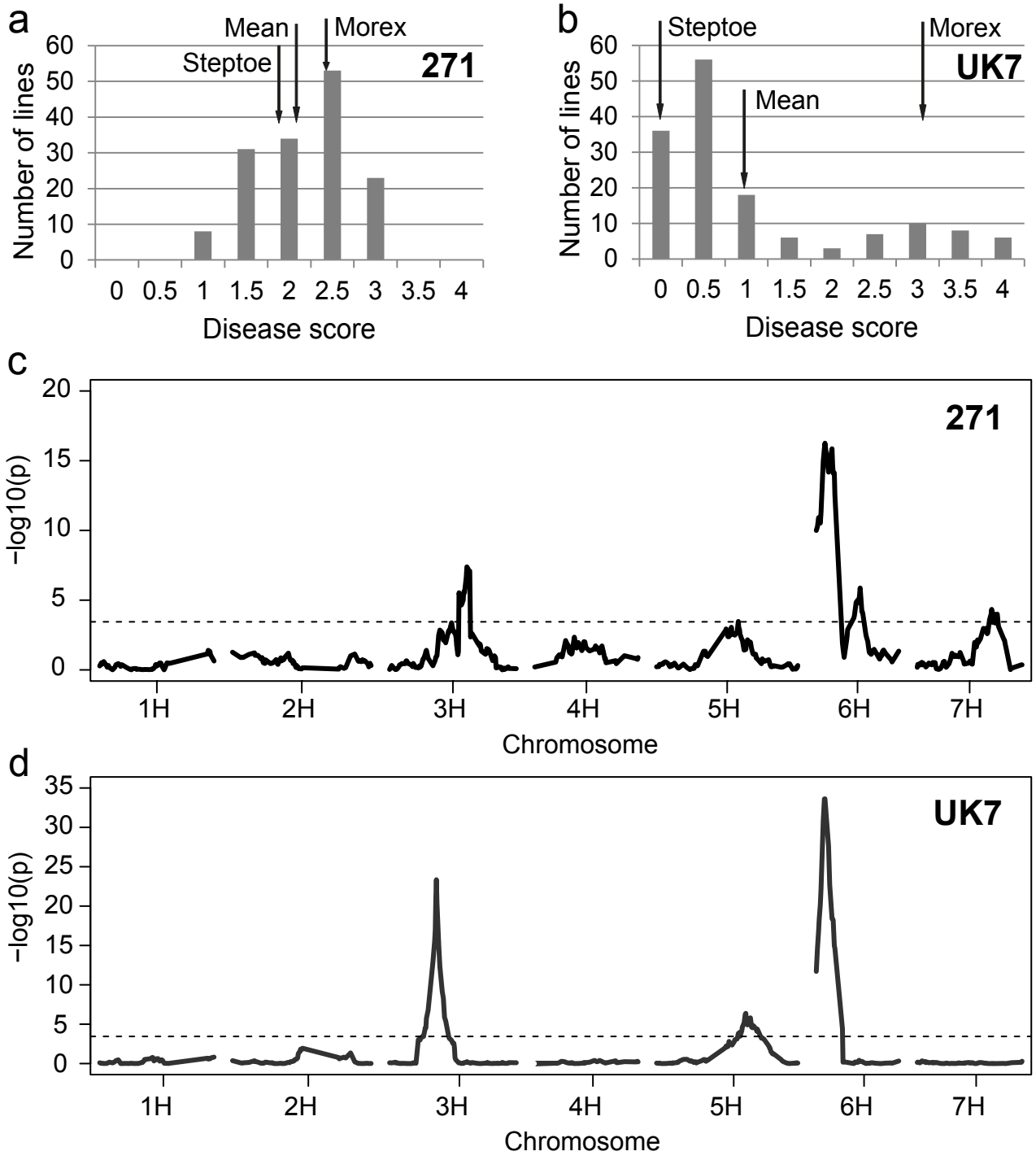

Fig. 1 Response of 140 Steptoe x Morex DH lines to *Rhynchosporium commune* isolates **a** 271 and **b** UK7. The Jackson and Webster (1976) scale extended by half steps was used. Vertical arrows indicate disease scores of parental cultivars Steptoe and Morex, and mean disease scores. A single environment QTL scan fc disease severity using *R. commune* isolates **c** 271 and **d** UK7 on Steptoe x Morex DH population. The horizontal dashed line represents the significance threshold corresponding to a genome-wide error rate of 0.05.

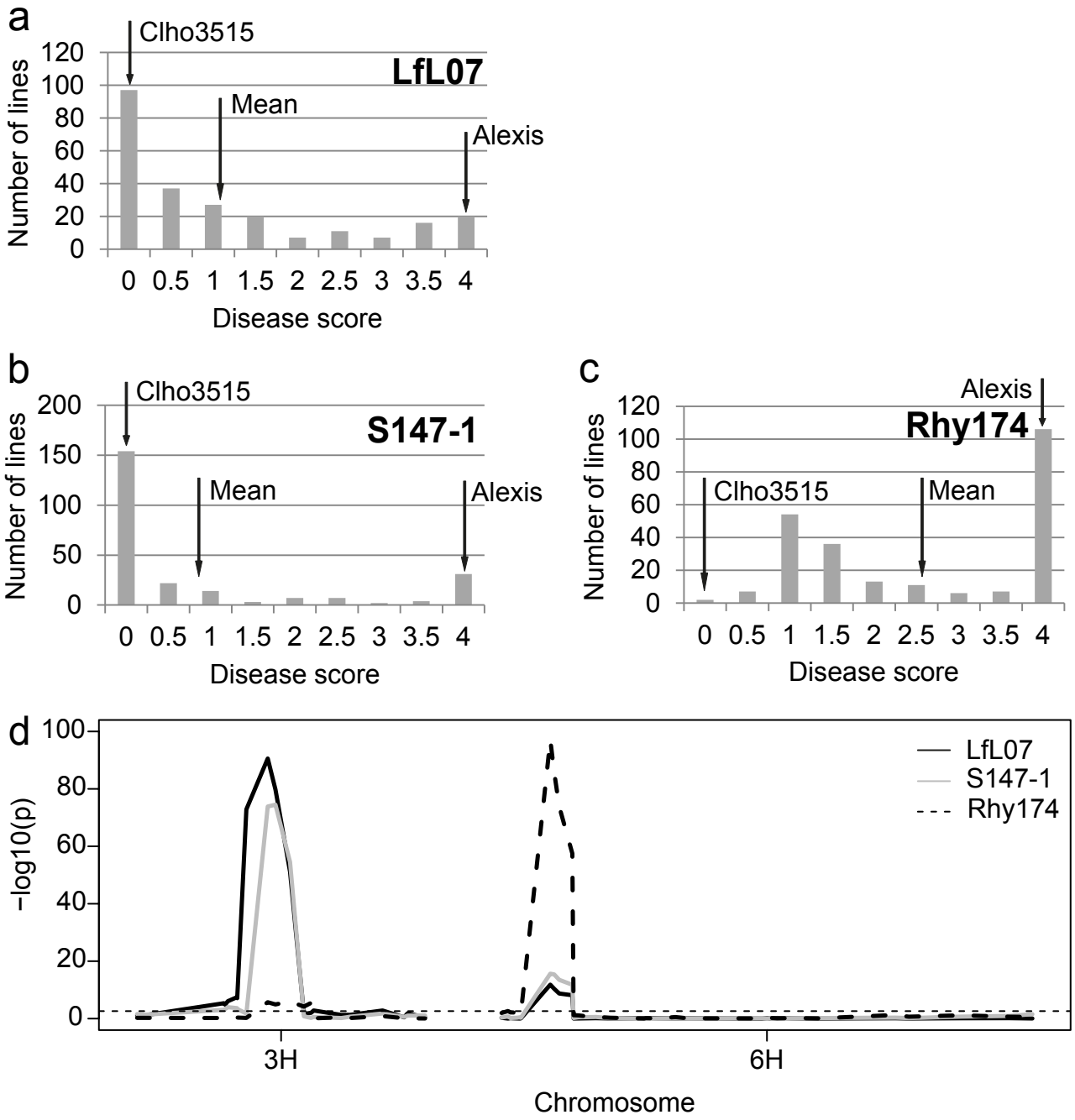

Fig. 2 Response of 238, 239 and 238 CIho 3515 x Alexis DH lines to *Rhynchosporium commune* isolates **a** LfL07, **b** S147-1and **c** Rhy174 respectively. The Jackson and Webster (1976) scale extended by half steps was used. Vertical arrows indicate disease scores of parents CIho 3515 and Alexis, and mean disease scores. **d** QTL on chromosomes 3H and 6H of CIho 3515 x Alexis DH population for *R. commune* isolates LfL07, S147-1 and Rhy174. The horizontal dashed line represents the significance threshold corresponding to an overall error rate of 0.05.

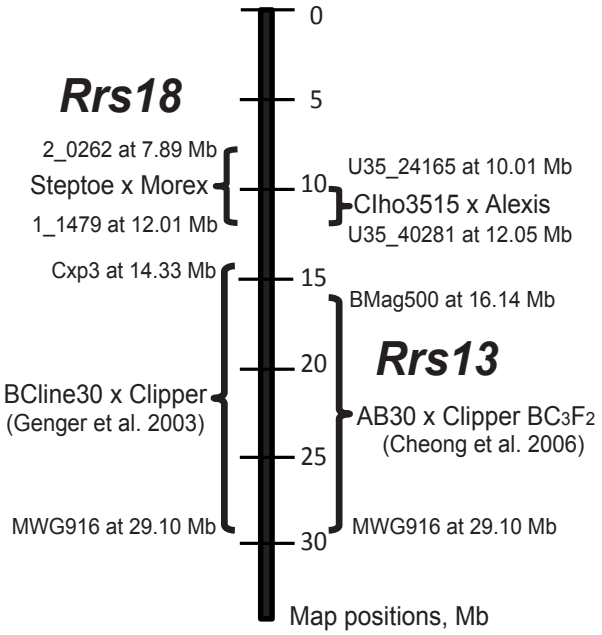

Fig. 3 Positions of marker sequences on 6HS pseudomolecule of Morex genome assembly v4. The diagram shows the first 30 Mb of the Morex 6HS pseudomolecule. Brackets show the flanking markers for QTL identified for resistance to rhynchosporium, and the parental lines used in QTL mapping. The physical position of *Rrs13* was identified using flanking markers Cxp3 and MWG916 from BC line 30 x Clipper population (Genger et al. 2003) and flanking markers BMag500 and MWG916 from AB30 x Clipper BC_3F_2 population (Cheong et al. 2006). The resistance QTL identified in Steptoe x Morex and CIho 3515 x Alexis DH populations at roughly the same position distal to *Rrs13* have been assigned as the *Rrs18* locus.

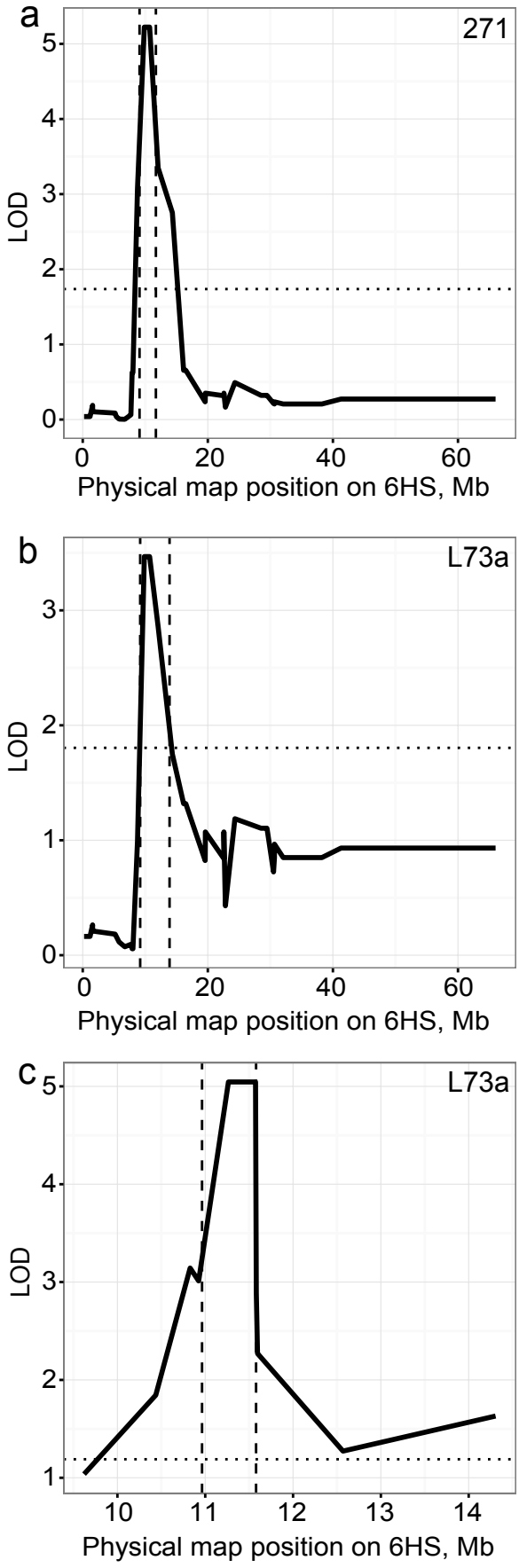

Fig. 4 Fine mapping of rhynchosporium severity on selected Steptoe x Morex BC_1S_2 lines using *Rhynchosporium commune* isolates **a** 271, **b** L73a. Further fine mapping was carried out with additional Steptoe x Morex BC_1S_2 lines using *R. commune* isolate L73a (**c**). The horizontal dashed line shows the Logarithm of the odds (LOD) corresponding to an overall error rate of 0.05.

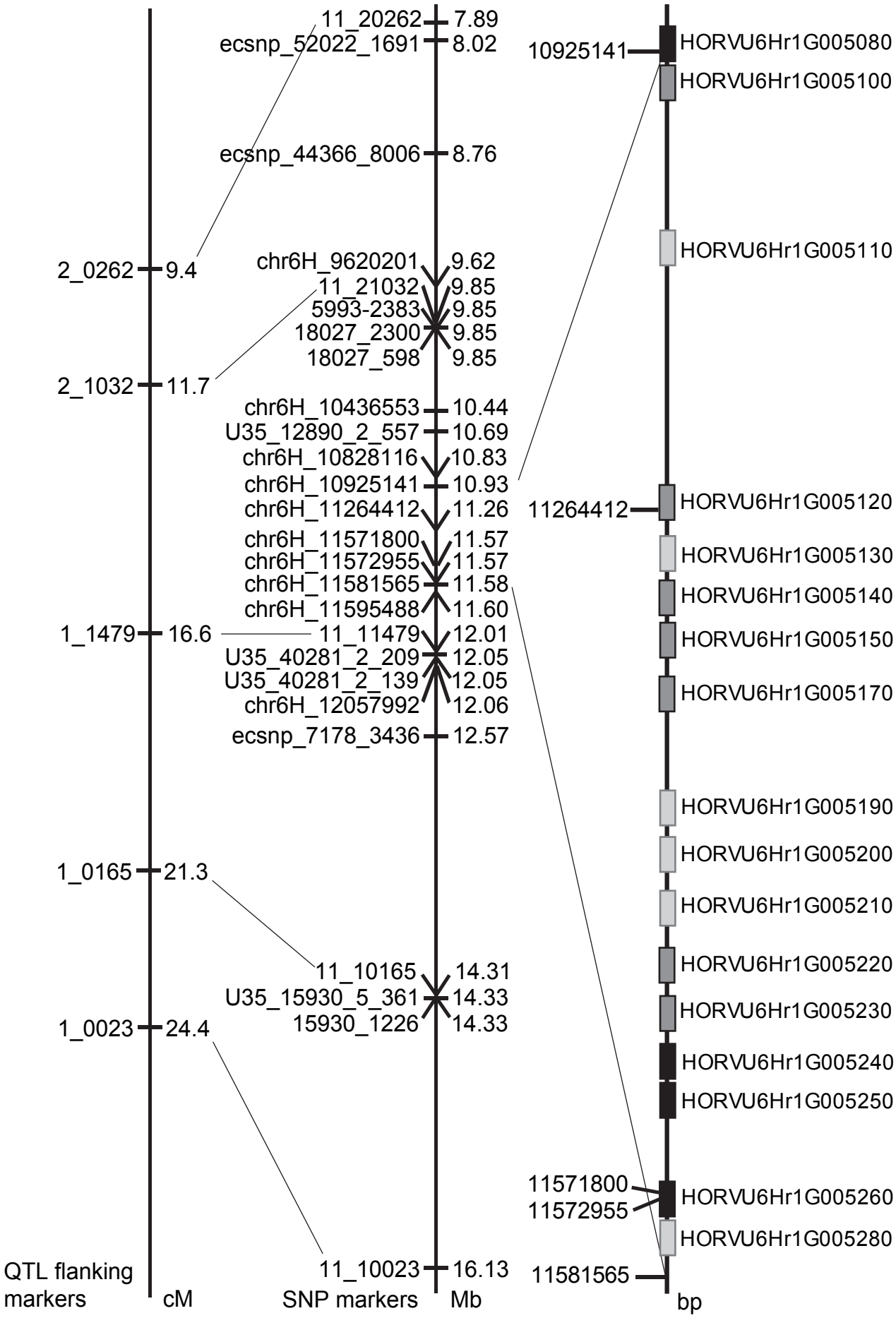

Fig. 5 Partial genetic map of chromosome 6H, linked to physical map, depicting the markers used for fine mapping of *Rrs18* in Steptoe x Morex BC_1 population, and gene content within *Rrs18* interval based on the latest Morex genome annotation (Mascher et al. 2017) **(**not drawn to scale). Black rectangles represent genes transcribed in leaves of CIho3515 and Alexis, dark and light grey rectangles represent remaining high and low confidence genes respectively.